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Sabatini et al.

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(54) **MAMMALIAN PROTEINS THAT BIND TO FKBP12 IN A RAPAMYCIN-DEPENDENT FASHION**

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(51) **Int. Cl.**[7] **C12Q 1/68**

(52) **U.S. Cl.** **435/6**; 536/23.1; 536/23.4; 536/23.5; 536/24.3

(58) **Field of Search** 536/23.5, 23.4, 536/24.3, 23.1; 530/350; 435/69.1, 69.7, 91.1, 91.2, 6

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(57) **ABSTRACT**

A protein complex containing 245 kDa and 35 kDa components, designated RAFT1 and RAFT2 (for Rapamycin And FKBP12 Target) interacts with FKBP12 in a rapamycin-dependent manner. This interaction has the pharmacological characteristics expected from the observed in vivo effects of rapamycin: it occurs at low nanomolar concentrations of rapamycin and is competed by excess FK506. Sequences (330 amino acids total) of tryptic peptides derived from the affinity purified 245 kDa RAFT1 reveals striking homologies to the predicted products of the yeast TOR genes, which were originally identified by mutations that confer rapamycin resistance in yeast. A RAFT1 cDNA was obtained and found to encode a 289 kDa protein (2550 amino acids) that is 43% and 39% identical to TOR2 and TOR1, respectively.

12 Claims, 10 Drawing Sheets

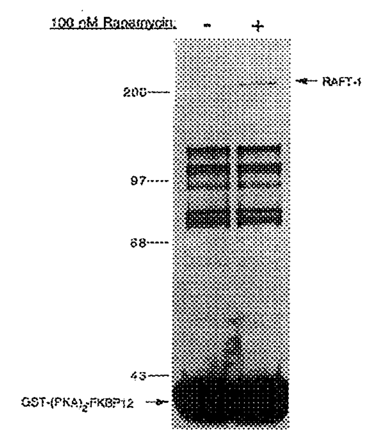

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FIG. 1

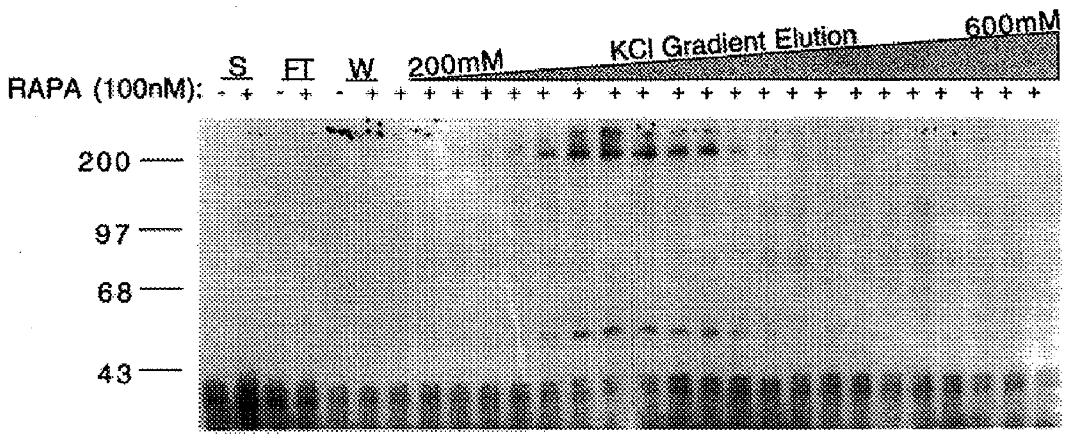
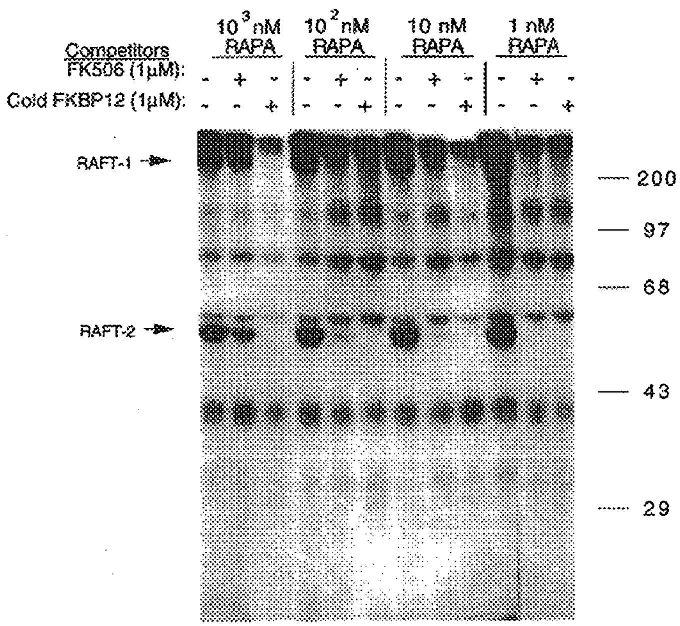
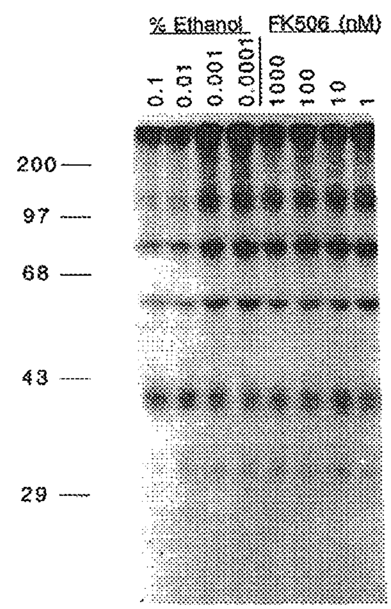

S
FT
W
200mM
KCl Gradient Elution
600mM
RAPA (100nM): - + - + - +
200
97
68
43

FIG. 2A



10^3 nM RAPA
10^2 nM RAPA
10 nM RAPA
1 nM RAPA
Competitors
FK506 (1μM):
Cold FKBP12 (1μM):
RAFT-1
RAFT-2
200
97
68
43
29

FIG. 2B



% Ethanol
FK506 (nM)
0.1
0.01
0.001
0.0001
1000
100
10
1
200
97
68
43
29

FIG. 3

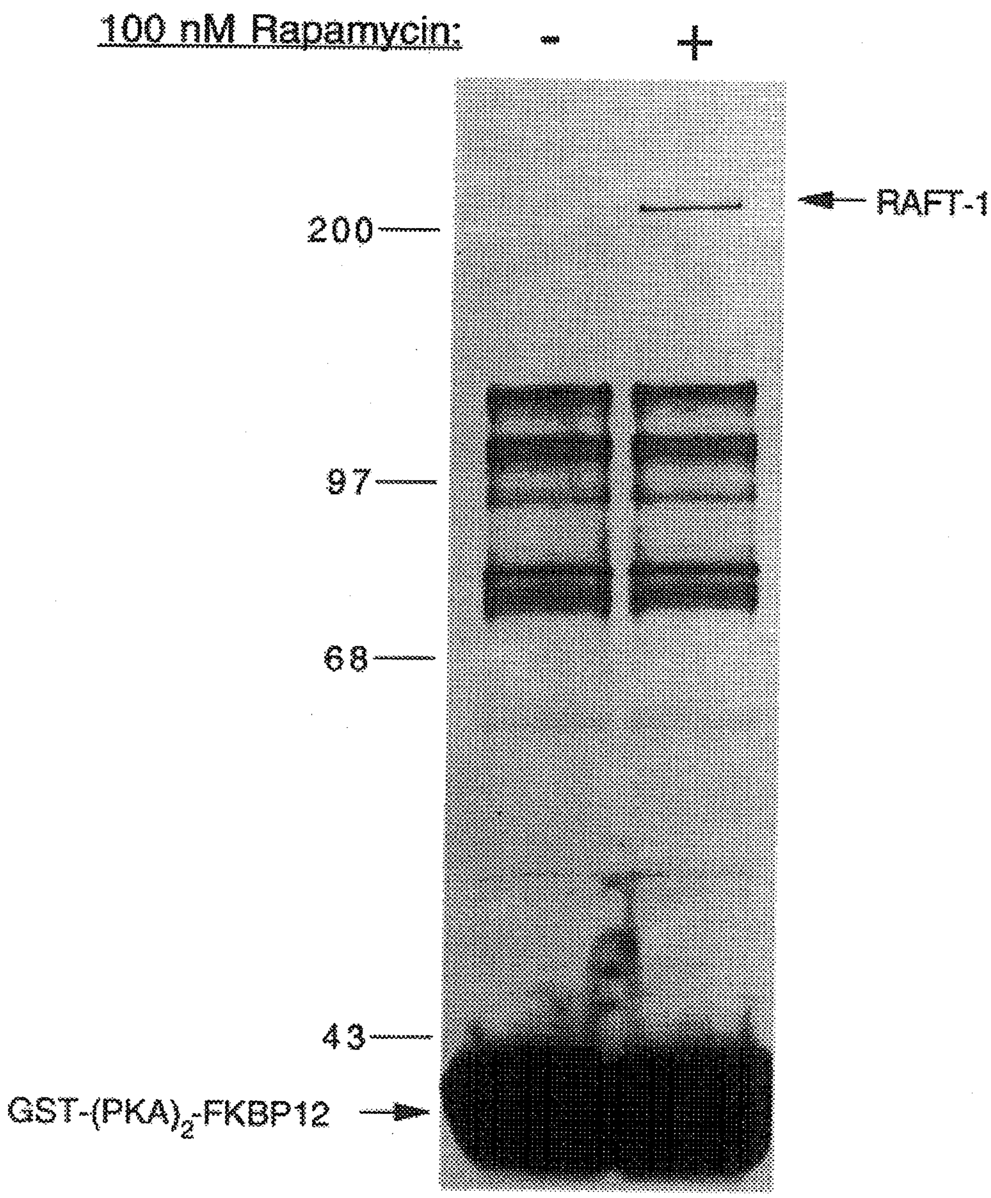


FIG. 4A

RAFT1	MLGTGPATATAGAATSSNVSVLOOFASGLKSRNEETRAKAAKELOHYVTME
TOR2	SAGHIGKISFVDSELDTTFSTLNLIFDKLKSDVPOERASGANELSTTLTSL
TOR1	TSSRFDGVVIGSNGDVNFKPILEKIFRELTSDYKEERKLASISLFDLLVSL
RAFT1	STRIGRFANYLRNLLPSSDPVVMEMASKAIGRLAMAGDTFTAEYVEFEVKR
TOR2	OT--SRLANYLRVLIPSSDIEVMRLAANTLGRLTVPGGTLTSDFVEFEVRT
TOR1	ET--SRLAGYLRGLIPSNDVEVMRLAAKTLGKLAVPGGTYTSDFVEFEIKS
RAFT1	AVWDPKOAIREGAVAALRACLILTTOREPKEMOKPOWYRHTFEEAEKGFDE
TOR2	PLRDAKLIIRLDAAVALGKCLTIIODRDPA--LGKOWFORLFOGCTHGLS-
TOR1	ALRDPHLVIRIDASITLAKCLSTLRNRDPO--LTSOWVORLATSCEYGFO-
RAFT1	DLMGFGTKPRHITPFTSFOAVOPOOSNALVGLLGYSSHOGLMGFGASPSPT
TOR2	---
TOR1	---
RAFT1	FTDTOYLODTMNHVLSCVKKEKER-----------------TAAFOALGL
TOR2	FTK-KYLDRIMVHYLR-----------YLKNIDMNAANNSDKPFILVSIGD
TOR1	FAG-KYLHOIMDNYLEILTNAPAKKIPHLKD---------DKPOILISIGD
RAFT1	GPGIOODI-KELLEPMLAVGLSPALTAVLYDLSROIPOLKKDIODGLLKMI
TOR2	GPAFAKHLNKDLLNLMLNCPMSDHMOETLMILNEKIPSLESTVNSRILNLL
TOR1	GPVLGKLLNRNILDLMFKCPLSDYMOETFOILTERIPSLGPKINDELLNLV
RAFT1	SDVASITLALRTLGSFEFEGHSLTOFVRHCADHFLNSEHKEIRMEAARTCS
TOR2	TDAOILIOCFKMLOLIHHO-YSLTEFVRLITISYIEHEDSSVRKLAALTSC
TOR1	NDIKIIIOAFRMLKNIKSR-FSLVEFVRIVALSYIEHTDPRVRKLAALTSC
RAFT1	LDERFDAHLAOAENLOALFVALNDOVFEIRELAICTVGRLSSMNPAFVMPF
TOR2	LGSNFDPOLAOPDNLRLLFMALNDEIFGIOLEAIKIIGRLSSVNPAYVVPS
TOR1	LNPCFDPOLAOPDNLRLLFTALHDESFNIOSVAMELVGRLSSVNPAYVIPS
RAFT1	KDPDPDPNPGVINNVLATIGELAOVSGLEMRKWVDELFVIIMDMLODSSLL
TOR2	Q----DASSAVASTALKVLGELSVVGGKEMTRYLKELMPLIINTFODOSNS
TOR1	Q----DTSSTVASTALRTIGELSVVGGEDMKIYLKDLFPLIIKTFODOSNS

FIG. 4B

```
LREMSOEESTRFYDOLNHHIFELVSSSDANERKGGILAIASLIGV-----EGGN    100
AREVSAEOFORFSNSLNNKIFELIHGFTSSEKIGGILAVDTLISFYLSTEELPN    169
EHELSIEEFOAISNDINNKILELVHTKKTNTRVGAVLSIDTLISFYAYTERLPN    157

ALEWL--GADRN-------EGRRHAAVLVLRELAISVPTFFFOOVOPFFDNIFV    196
CIDWLTLTADNNS-SSSKLEYRRHAALLIIKALADNSPYLLYPYVNSILDNIWV    271
CLEWLTASTEKNSFSSSKPDHAKHAALLIITALAENCPYLLYOYLNSILDNIWR    260

TLAKEKGMNRDDRIHGALLILNELVRISSMEGERLREEMEEITOOOLVHDKYCK    301
-------LNTNDSVHATLLVFRELLSLKA-------------------------    350
-------VNTLECIHASLLVYKEILFLKD-------------------------    339

KSTLVESRCCRDLMEEKFDOVCOWVLKCRSSKNSLIOMTILNLLPRLVAFRPSA    406
-----------PYLRDKYDDIYKSTMKYKEYKFDVIRREVYAILPLLAAFDPAI    384
-----------PFLNOVFDOMCLNCIAYENHKAKMIREKIYOIVPLLASFNPOL    373

LSVAVRSEFKVYLPRVLDIIRAALPPKDFAHKROKTVOVDATVFTCISMLARAM    493
IAFEVGSSISPYMTLILDNIREGLRTK-FKVRKO----FEKDLFYCIGKLACAL    472
IAYEVGPDIAPYVKOILDYIEHDLOTK-FKFRKK----FENEIFYCIGRLAVPL    463

SLVLMHKPLRHPGMPK--------------------GLAHOLASPGLTTLPEA    576
SISLSGEKFIO--------SNOYDFNNOFSIEKARKSRNOSFMKKTGESN-DDI    568
CSTLSGTPFIOPGSPMEIPS---------FSRERAREWRNKSILOKTGESN-DDN    559

RLLTPSIHLISGHAHVVSOTAVOV---VADVLSKLLVVGITDPDPDIRYCVLAS    678
DLFI---------KDDICKOTSVHALHSVSEVLSKLLMIAITDPVAEIRLEILOH    664
EIYV---------KDNICKOTSLHSLNTVSEVLSKLLAITIADPLODIRLEVLKN    655

LRKMLIOILTELEHSGIGRIKEOSARMLGHLVSNAPRLIRPYMEPILKALILKL    783
LRKTLLELLTOLKFSNMPKKKEESATLLCTLINSSDEVAKPYIDPILDVILPKC    769
IRKILLELLTKLKFSTSSREKEETASLLCTLIRSSKDVAKPYIEPLLNVLLPKF    760

AKROVALWTLGOLVASTGYVVEPYRKYPTLLEVLLNFLKTEONOGTRREAIRVL    888
FKRDAALTTLGOLAASSGYVVGPLLDYPELLGILINILKTENNPHIRRGTVRLI    870
FKREAALKALGOLAASSGYVIDPLLDYPELLGILVNILKTENSONIRROTVTLI    861
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FIG. 4C

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RAFT1  GLLGALDPYKHKVNIGMIDOSRDASAVSLSESKSSODSSDYSTSEMLVNMG
TOR2   GILGALDPYKHR---------EIEVT----SNSKSSVEONAPSIDIALLMOG
TOR1   GILGAIDPYROK---------EREVT----STTDISTEONAPPIDIALLMOG

RAFT1  VMPTFLNVIRVCDGAIREFLFOOLGMLVSFVKSHIRPYMDEIVTLMREFWV
TOR2   IIPGIILVMRSCPPSOLDFYFOOLGSLISIVKOHIRPHVEKIYGVIREFFP
TOR1   IIPTILDVMRTCSOSLLEFYFOOLCSLIIIVROHIRPHVDSIFOAIKDFSS

RAFT1  AAIOLFGANLDDYLHLLLPPIVKLFDAPEVPLPSRKAALETVDRLTESLDF
TOR2   KSLVTFGPNLEDYSHLIMPIVVRMTEYSAGSL--KKISIITLGRLAKNINL
TOR1   RLLESFGPNLEGYSHLITPKIVOMAEFTSGNL--ORSAIITIGKLAKDVDL

RAFT1  RHRINHORYDVLICRIVKGYTLA---------DEEEDPLIYOHRMLRSSOGD
TOR2   RNRIOHSVYDOLVNKLLNNECLPTNIIFDKENEVPERKNYEDEMO------
TOR1   KKHIOHTIYDDLTNRILNNDVLPTKIL---EANTTDYKPAE-OMEAADAG-

RAFT1  PSLRSCWALAOAYNPMARDLFNAAFVSCWSELNEDOODELIRSIELALTS-
TOR2   ACLRSCSSLVSVYYPLARELFNASFSSCWVELOTSYOEDLIOALCKALSSS
TOR1   HALRACSNLASMYYPLAKELFNTAFACVWTELYSOYOEDLIGSLCIALSSP

RAFT1  LEFOKGPTPAILESLISINNKLOOPEAASGVLEYAMKHFGELEIOATWYEK
TOR2   VEFLEEPKNSTIEALISINNOLHOTDSAIGILKHAOOH-NELOLKETWYEK
TOR1   IKFIKEPENSTIESLISINNOLNOTDAAIGILKHAOOH-HSLOLKETWFEK

RAFT1  ETOAKMARMAAAAAWGLGOWDSMEEYTCMIPRDTHDGAFYRAVLALHODLF
TOR2   EVKKAMAPLAAGAAWGLEOWDEIAOYTSVMKSOSPDKEFYDAILCLHRNNF
TOR1   OTKKLIAPLAAGARWGLGEWDMLEOYISVMKPKSPDKEFFDAILYLHKNDY

RAFT1  -ERREIIROIWWERLOGCORIVEDWOKILMVRSLVVSPHEDMRTWLKYASL
TOR2   SDKRLTMRETWNTRLLGCOKNIDVWORILRVRSLVIKPKEDAOVRIKFANL
TOR1   SEKKLHYONLWTKRLLGCOKNVDLWORVLRVRSLVIKPKODLOIWIKFANL

RAFT1  IDAFOHMOHF----------------VOTMOOOAOHAIATEDOOHKOELHK
TOR2   DEALKOLINFTSRMAHDLGLDPNNMIAOSVPOOSKRV-----PRHVEDYTK
TOR1   KEALNHLIGFTSRLAHDLGLDPNNMIAOSVKLSSAST-----APYVEEYTK
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FIG. 4D

```
NLPL-DEFYPAVSMVALMRIFRDOSLSHHHTMVVOAITFIFKSLGLKCVOFLPQ   992
VSPSNDEYYLTVVIHNLMKILNDPSLSIHHTAAIOAIMHIFONLGLRCVSFLDO   963
MSPSNDEYYTTVVIHCLLKILKDPSLSSYHTAVIOAIMHIFOTLGLKCVSFLDO   954

MNTSIOSTIILLIEOIVVALGGEFKLYLPOLIPHMLRVFMHDNSOGRIVSIKLL  1097
I-IKLOITIISVIESISKALEGEFKRFVPETLTFFLDILENDOSNKRIVPIRIL  1067
V-AKLOITLVSVIEAISKALEGEFKRLVPLTLTLFLVILENDKSSDKVLSRRVL  1058

TDYASRIIHPIVRTLDO--SPELRSTAMDTLSSLVFOLGKKYOIFIPMVNKVLV  1200
SEMSSRIVOALVRILNNGDR-ELTKATMNTLSLLLOLGTDFVVFVPVINKALL  1169
FEMSSRIVHSLLRVLSSTTSDELSKVIMNTLSLLLIOMGTSFAIFIPVINEVLM  1161

ALASGPVETGPMKKLHVSTINLOKAWGAARRVSKDDWLEWLRRLSLELLKDSSS  1297
------------VTKLPVNONILKNAWYCSOOKTKEDWOEWIRRLSIOLLKESPS  1257
------------VAKLPINOSVLKSAWNSSOORTKEDWOEWSKRLSIOLLKESPS  1250

ODIAEVTOTLLNLAEFMEHSDKGPLPLRDDNGIVLLGERAAKCRAYAKALHYKE  1401
ENPPEIYOMLLNLVEFMEHDDK-PLPIP----IHTLGKYAOKCHAFAKALHYKE  1357
LNPPEIHOTLLNLVEFMEHDDK-ALPIP----TOSLGEYAERCHAYAKALHYKE  1350

LHEWEDALVAYDKKMDTNKDDPELMLGRMRCLEALGEWGOLHOOCCEKWTLVND  1506
LORWEDALAAYNEKEAAGEDSVEVMMGKLRSLYALGEWEELSKLASEKWGTAKP  1461
LERWEDALHAYNEREKAGDTSVSVTLGKMRSLHALGEWEOLSOLAARKWKVSKL  1454

SLAOOCIDKARDLLDAELTAMAGESYSRAYGAMVSCHMLSELEEVIOYKLVP--  1609
KKAEVHIFNARDLLVTELSALVNESYNRAYNVVVRAOIIAELEEIIKYKKLPON  1566
DNASKHILNARDLLVTEISALINESYNRAYSVIVRTOIITEFEEIIKYKOLPPN  1559

CGKSGRLALAHKTLVLLL--GVDPSROLDHP-LPTVHPOVTYAYMKNMWKSARK  1710
CRKSGRMALAKKVLNTLLEETDDP----DHPNTAKASPPVVYAOLKYLWATGLO  1667
CRKSGRMRLANKALNMLLEGGNDP----SLPNTVKAPPPVVYAOLKYIWATGAY  1660

LMARCFLKLGEWOLNLOGINESTIPK-VLOYYSAATEHDRSWYKAWHAWAVMNF  1798
LLARCFLKOGEWRVCLOPKWRLSNPDSILGSYLLATHFDNTWYKAWHNWALANF  1767
LLARCFLKOGEWRIATOPNWRNTNPDAILGSYLLATHFDKNWYKAWHNWALANF  1760
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FIG. 4E

```
RAFT1   EAVLHYKHONOARDEKKKLRHASGANITNATTTATTAASAAAATSTEGSNS
TOR2    EVISMLTSVSK---KKOE-----------GSDASSVTDIN-EFDNGMIGVNT
TOR1    EVISMVOEETKLNGGKND-----------DDDDTAVNNDNVRIDGSILGSGS

RAFT1   LRVLTLWFDYGHWPDVNEALVEGVKAIOIDTWLOVIPOLIARIDTPRPLVG
TOR2    LRLLTLWFTFGGIPEATOAMHEGFNLIOIGTWLEVLPOLISRIHOPNOIVS
TOR1    LRLLTLLFNFGGIKEVSOAMYEGFNLMKIENWLEVLPOLISRIHOPDPTVS

RAFT1   AMMVSEELIRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMME
TOR2    AELVSHELIRMAVLWHEOWYEGLDDASROFFGEHNTEKMFAALEPLYEMLK
TOR1    AELVSHELIRVAVLWHELWYEGLEDASROFFVEHNIEKMFSTLEPLHKHLG

RAFT1   OLPOLTSLELOYVSPKLLMCRDLELAVPGTYDPN-OPIIRIOSIAPSLOVI
TOR2    OLPOLOTLELOHVSPKLLSAHDLELAVPGTRASGGKPIVKISKFEPVFSVI
TOR1    OIPOLOTLDLOHVSPOLLATHDLELAVPGTYFP-GKPTIRIAKFEPLFSVI

RAFT1   KNLSIORYAVIPLSTNSGLIGWVPHCDTLHALIRDYREKKKILLNIEHRIM
TOR2    RHLDIOOYPAIPLSPKSGLLGWVPNSDTFHVLIREHREAKKIPLNIEHWVM
TOR1    RHLDIOOYPAIPLSPKSGLLGWVPNSDTFHVLIREHRDAKKIPLNIEOWVM

RAFT1   SLAVMSMVGYILGLGDRHPSNLMLDRLSGKILHIDFGDCFEVAMTREKFPE
TOR2    SLAVMSMTGYILGLGDRHPSNLMLDRITGKVIHIDFGDCFEAAILREKFPE
TOR1    SLAVMSMTGYILGLGDRHPSNLMLDRITGKVIHIDFGDCFEAAILREKYPE

RAFT1   NWRLMDTNAKGNKRSRTRTDSYSAGOSVEILDGVELGEPAHK---KTGTTV
TOR2    NW------------------------------GFDL--PTKKIEEETGIOL
TOR1    HW------------------------------GFDL--PPOKLTEOTGIPL

RAFT1   DTLDVPTOVELLIKOATSHENLCOCYIGWCPFW
TOR2    NDLDVPEOVDKLIOOATSVENLCOHYIGWCPFW
TOR1    NELDVPEOVDKLIOOATSIERLCOHYIGWCPFW
```

FIG. 4F

```
ESEAESNESSPTPSPLQKKVTEDLSKTLLLYTVPAVQGFFRSISLSRGNNLQDT  1903
---FDAKEVHYSSNLIHRHV-------------IPAIKGFFHSISLSESSSLQDA  1843
---LTINGNRYPLELIQRHV------------VPAIKGFFHSISLLETSCLQDT  1840

RLIHQLLTDIGRYHPQALIYPLTVASKSTTTARHNAANKILKNMCEHSNTLVQQ  2008
RSLLSLLSDLGKAHPQALVYPLMVAIKSESLSRQKAALSIIEKMRIHSPVLVDQ  1948
NSLLSLLSDLGKAHPQALVYPLTVAIKSESVSRQKAALSIIEKIRIHSPVLVNQ  1945

RGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISK  2113
RGPETLREISFQNSFGRDLNDAYEWLMNYKKSKDVSNLNQAWDIYYNVFRKIGK  2053
NEPQTLSEVSFQKSFGRDLNDAYEWLNNYKKSKDINNLNQAWDIYYNVFRKITR  2050

TSKQRPRKLTLMGSNGHEFVFLLKGHEDLRQDERVMQLFGLVNTLLANDPTSLR  2217
SSKQRPRKFCIKGSDGKDYKYVLKGHEDIRQDSLVMQLFGLVNTLLQNDAECFR  2158
SSKQRPRKFSIKGSDGKDYKYVLKGHEDIRQDSLVMQLFGLVNTLLKNDSECFK  2154

LRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLWLKSPSSEVWFDRRTNYTR  2322
LQMAPDYDNLTLLQKVEVFTYALNNTEGQDLYKVLWLKSRSSETWLERRTTYTR  2263
LQMAPDYENLTLLQKIEVFTYALDNTKGQDLYKILWLKSRSSETWLERRTTYTR  2259

KIPFRLTRMLTNAMEVTGLDRNYRTTCHTVMEVLREHKDSVMAVLEAFVYDPLL  2427
KVPFRLTRMLTYAMEVSGIEGSFRITCENVMKVLRDNKGSLMAILEAFAFDPLI  2368
KVPFRLTRMLTYAMEVSGIEGSFRITCENVMRVLRDNKESLMAILEAFALDPLI  2364

PE-SIHSFIGDGLVKPEAL-------------NKKAIQIINRVRDKLTGRDFSHD  2517
PVMNANELLSNGAITEEEVQRVENEHKNAIRNARAMLVLKRITDKLTGNDIRRF  2441
PLINPSELLRKGAITVEEAANMEAEQQNETRNARAMLVLRRITDKLTGNDIKRF  2437

                                                        2550
                                                        2474
                                                        2470
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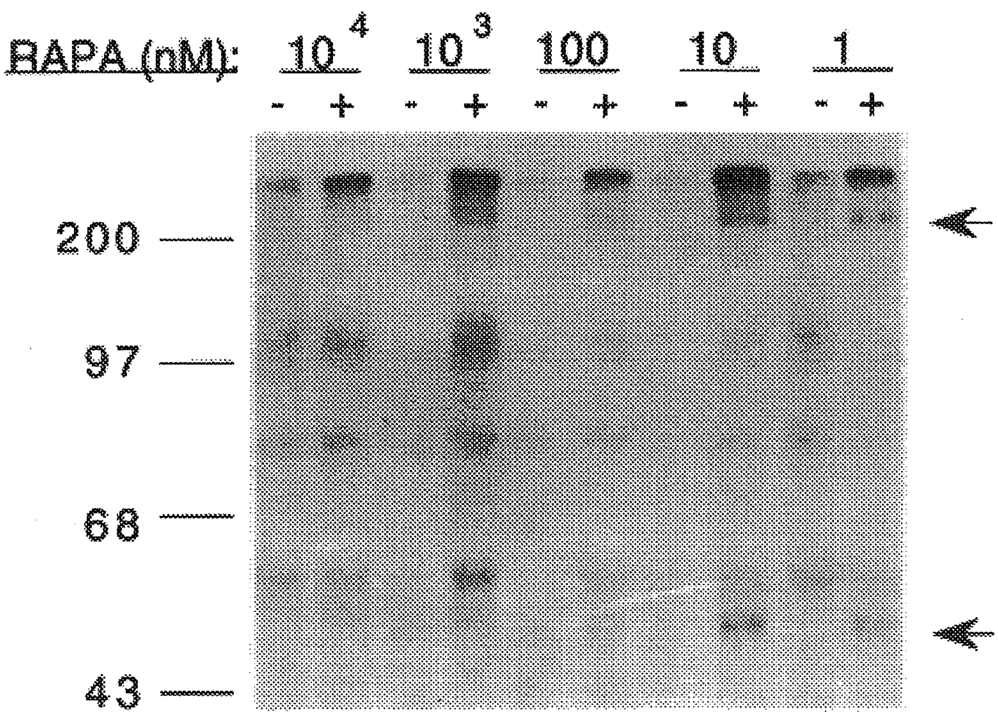
FIG. 5



MAMMALIAN PROTEINS THAT BIND TO FKBP12 IN A RAPAMYCIN-DEPENDENT FASHION

This application is a continuation-in-part of application Ser. No. 08/265,967, filed on Jun. 27, 1994.

This invention was made with government support under MH18501, DA00266, and DA00074, awarded by the National Institutes of Health. The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The natural products cyclosporin A, FK506, and rapamycin are potent immunosuppressants with realized or potential clinical applications in the prevention of graft rejection after organ transplantation and the treatment of autoimmune disorders (Borel, 1986; Kino et al., 1987; Martel et al., 1977). These drugs act by inhibiting intermediate steps in the signaling pathways that effect the T-cell response to antigen (for reviews see, Fruman et al., 1994; Kunz and Hall, 1993; Liu, 1993; Schreiber, 1991). This makes them useful probes for identifying the components of those pathways and determining their physiological roles.

The immunosuppressants interact with the immunophilins, which are small, soluble, receptor proteins that mediate their actions. Cyclosporin A (a cyclical undecapeptide) binds to cyclophilin A, whereas FK506 and rapamycin (two related macrolide antibiotics) bind to a distinct receptor protein, FKBP12 (Handschumacher et al., 1984; Harding et al., 1989; Siekierka et al., 1989). Though cyclophilin and FKBP12 differ markedly in amino acid sequence, both immunophilins have peptidyl-prolyl cis-trans isomerization (rotamase) activity, which is inhibited by their respective ligands (for review, see Heitman et al., 1992). However, this inhibition does not appear to explain the effects of the immunosuppressants (Bierer et al., 1990a, b; Tropschug et al., 1989). Instead, the action of cyclosporin A and FK506 derives from the binding of the drug-receptor complexes to the calcium-activated protein phosphatase, calcineurin (Liu et al., 1991). This association inhibits the catalytic activity of the phosphatase, which is required for the Ca^{++}-dependent initial step in the activation of the T-lymphocyte via the T-cell receptor (Flanagan et al., 1991; Kronke et al., 1984).

On the other hand, rapamycin appears to block a later, Ca^{++}-independent stage in the T-cell response. This drug selectively inhibits the IL-2 stimulated G1 to S cell-cycle transition that initiates T-cell proliferation (Dumont et al., 1990b). Although this inhibition has been correlated with the decreased activity of the 70 kDa S6 kinase ($pp70^{S6K}$), a known downstream effector of the IL-2 receptor, the FKBP12-rapamycin complex does not appear to interact directly with this kinase (Chung et al., 1992; Kuo et al., 1992). Similarly, in T-cells and other cell types, rapamycin blocks progression of the cell cycle by preventing the activation of the cyclin-dependent kinases $p33^{cdk2}$ and $p34^{cdc2}$, but an association of the drug-immunophilin complex with the kinases or their respective cyclins has not been demonstrated (Albers et al., 1993; Jayaraman and Marks, 1993; Morice et al., 1993).

In the budding yeast *S. cerevisiae,* rapamycin also causes an arrest in the G1 phase of the cell cycle through its binding to a highly conserved FKBP12 homologue (Heitman et al., 1991b). The toxicity of the drug for yeast cells has allowed, through genetic selection, the identification of two homologous genes, which, when mutated, render the cells rapamycin-resistant (Heitman et al., 1991a). This led to the proposal that the products of these genes, which show some amino acid homology to the catalytic domain of the p110 subunit of PI-3 kinase, are the Targets Of Rapamycin and hence to the designation of the genes as TOR1 and TOR2 (Kunz et al., 1993). Direct support for this proposal, however, has not been presented and how the TOR gene products confer sensitivity to rapamycin remains to be elucidated. Alternatively, it has been suggested that in the signaling pathway blocked by rapamycin, the TOR proteins, like the S6 kinase and the cyclin-dependent kinases, lie downstream from the direct target of the FKBP12-rapamycin complex (Albers et al., 1993; Helliwell et al., 1994). This model assumes that the TOR mutations lead to the constitutive activation of the TOR1 and TOR2 proteins.

Besides binding to calcineurin in a FK506-dependent manner, FKBP12 can also interact with calcium-channel proteins, the ryanodine receptor, which mediates calcium induced calcium released (Jayaraman et al., 1992; Timerman et al., 1993) and the inositol 1,4,5,-triphosphate (IP_3) receptor (A. Cameron, A. Kaplin, D. Sabatini, J. Steiner, S. Snyder, unpublished). These associations do not require FK506 or rapamycin; indeed these drugs dissociate the FKBP12-channel complex.

There is a need in the art to identify, isolate, and purify the mammalian cellular proteins that interact with FKBP12 only in the presence of rapamycin. Such proteins play a role in immunological, neurological, and cell cycle functions.

SUMMARY OF THE INVENTION

It is an object of the invention to provide isolated, purified cDNA molecules encoding rapamycin and FKBP target molecules.

It is another object of the invention to provide fusion proteins comprising rapamycin and FKBP targets.

It is still another object of the invention to provide an isolated and purified rapamycin and FKBP target molecule.

It is still another object of the invention to provide an expression construct which directs synthesis in a cell of an RNA molecule which inibits expression of a rapamycin and FKBP target molecule.

It is yet another object of the invention to provide isolated, purified cDNA molecules which are complementary to genes encoding rapamcyin and FKBP target molecules.

It is an object of the invention to provide a method of screening for potential therapeutic agents.

It is another object of the invention to provide a method of purifying a rapamycin and FKBP target molecule.

It is still another object of the invention to provide a method of isolating DNA sequences which code for rapamycin and FKBP target molecules.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment of the invention an isolated, purified cDNA molecule is provided which encodes RAFT1, a protein having the amino acid sequence shown in SEQ ID NO:2.

In another embodiment of the invention a fusion protein comprising the amino acid sequence shown in SEQ ID NO:2 is provided.

In yet another embodiment of the invention an isolated and purified RAFT1 protein having the amino acid sequence shown in SEQ ID NO:2 is provided. Also provided is an isolated and purified RAFT2 protein, having an apparent molecular weight on SDS polyacrylamide gels of 35 kDa. Also provided is an isolated and purified mammalian RAFT

protein which is free of proteins which do not bind to rapamycin and FKBP12. Also provided is a mammalian RAFT protein prepared by the process of:

contacting a preparation of mammalian proteins with FKBP12 in the presence of rapamycin;

isolating mammalian proteins which bind to FKBP12 in the presence of rapamycin from those mammalian proteins which do not bind; and

dissociating bound mammalian proteins from FKBP12 to provide a mammalian RAFT protein.

In still another embodiment of the invention an expression construct is provided. The expression construct comprises a promoter operably linked to at least 20 nucleotides of the antisense strand of RAFT1 cDNA, said expression construct directing synthesis in a cell of an RNA molecule which is complementary to RAFT1 RNA.

In another embodiment of the invention an isolated, purified cDNA molecule comprising at least 20 nucleotides of the sequence as shown in SEQ ID NO:1 is provided.

In yet another embodiment of the invention a method of screening substances for potential as therapeutic agents is provided. The method comprises the steps of:

contacting a substance to be tested with three components: (a) FKBP12, (b) rapamycin, and (c) a protein selected from the group consisting of RAFT1 and RAFT2;

determining the amount of one of said components bound to the other components in the presence and absence of said substance; a substance which increases or decreases the amount of said component bound being a potential therapeutic agent.

In one embodiment of the invention a method of purifying a mammalian RAFT protein is provided. The method comprises the steps of:

contacting a preparation of mammalian proteins with FKBP12 in the presence of rapamycin;

isolating mammalian proteins which bind to FKBP12 in the presence of rapamycin from those mammalian proteins which do not bind;

dissociating bound mammalian proteins from FKBP12 to provide a mammalian RAFT protein.

In another embodiment of the invention methods of isolating mammalian RAFT DNA sequences are provided. One of the methods comprises:

probing a library of mammalian DNA sequences with a probe which comprises at least 15 contiguous nucleotides selected from the sequence shown in SEQ ID NO:1.

Another of the methods comprises:

amplifying a DNA sequence using at least one primer which comprises at least 10 contiguous nucleotides selected from the sequence shown in SEQ ID NO:1.

These and other embodiments of the invention provide the art with potent tools for identifying drugs useful in the treatment of immunological, neurological, and cell cycle-related diseases and defects.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows partial purification of the FKBP12-rapamycin target proteins from brain cytosol by heparin column chromatography. A cytosolic fraction prepared from a rat brain homogenate was applied to a heparin column. The material that remained bound to the column after washing with 5 column volumes of wash buffer containing 200 mM KCl, was eluted with a linear gradient from 200 mM to 600 mM KCl in homogenization buffer. Aliquots of the crude cytosol (S), the column flow through (FT), and the wash (W) were tested in the crosslinking assay with (+) or without (−) rapamycin (100 nM). Every other fraction eluted from the heparin column was tested in the crosslinking assay in the presence of 100 nM rapamycin. No rapamycin specific crosslinked products are visible in the crude cytosol because of the high concentrations of endogenous FKBP12 present in the initial sample.

FIG. 2 shows FK506 and unlabeled FKBP12 prevent the rapamycin-dependent association of ^{32}P-FKBP12 to the target proteins.

FIG. 2A) The heparin column eluate containing the RAFTs was tested in the crosslinking assay at the indicated concentrations of rapamycin with or without the addition of 1 μM FK506 or 1 μM FKBP12. FIG. 2B) Neither FK506 alone nor the ethanol vehicle induce crosslinking of FKBP12 to RAFT. The heparin eluate containing RAFT was tested in the crosslinking assay with the indicated concentrations of FK506 or ethanol. This experiment was repeated twice with identical results.

FIG. 3 shows purification of RAFT1 with a FKBP12-rapamycin affinity column. RAFT enriched fractions eluting from the heparin column between 300 and 450 mM KCl, were incubated in the presence (+) or absence (−) of 100 nM rapamycin with GST-(PKA)2-FKBP12 fusion protein (20 μg) immobilized on glutatlione agarose beads. The material that remained associated with the beads after extensive washes was analyzed by SDS-PAGE (8%) and silver staining. RAFT1 is present only in the sample treated with rapamycin. The large band at 36 kDa is the GST-FKBP12 fusion protein.

FIG. 4 shows the alignment of RAFT1 amino acid sequence with the predicted amino acid sequences of TOR2 (SEQ ID NO:4) and TOR1 (SEQ ID NO:3).

The alignment was maximized by introducing insertions marked by dashes. Sequences in RAFT1 identical to TOR2 and/or TOR1 are indicated with gray shading. The sequences of tryptic peptides obtained by microsequencing are indicated with a line above the RAFT1 sequence. Sequences used to design primers for PCR are indicated with an arrow above the residues (direction indicate sense or antisense). The PKC site conserved between RAFT1, TOR1 and TOR2 is boxed.

FIG. 5 shows rapamycin-dependent crosslinking of FKBP12 to two PC12 cell cytosolic proteins of approximate molecular weight 245 kDa and 35 kDa.

^{32}P-labeled FKBP12 (10^5 cpm) was incubated with cytosolic fractions from PC12 cells with or without the indicated concentration of rapamycin for 1 hr. at 4° C. The crosslinker DSS was then added and the incubation continued for 40 minutes before processing for SDS-PAGE (4%–12% gradient) and autoradiography. The arrows indicate the two Lands that appear only in the presence of rapamycin. This experiment was repeated three times with identical results.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

We have isolated and identified proteins, which we designate RAFT1 and RAFT2, that interact with the FKBP12-rapamycin complex. Rapamycin-induced binding of FKBP12 to RAFT1 occurs at drug concentrations as low as 1 and 10 nM, resembling pharmacological potency in vivo (Bierer et al., 1990a; Dumont et al., 1990a). FK506 and rapamycin bind with similar affinities to the same binding site on FKBP12 and antagonize each others' actions in vivo (Bierer et al., 1990a; Dumont et al., 1990b). Consistent with

these facts, FK506 does not induce interactions between FKBP12 and RAFT1 but, instead, prevents the rapamycin-mediated effect. Since rapamycin has pleiotropic effects on a wide variety of cell types, the target of its complex with FKBP12 is likely to be an early participant in several signal transduction pathways.

We have also isolated and purified a cDNA molecule which encodes RAFT1. The nucleotide sequence of RAFT1 is shown in SEQ ID NO:1. The predicted amino acid sequence of the protein, which exactly corresponds to the empirically determined amino acid sequences of tryptic peptides of RAFT1, is shown in SEQ ID NO:2. The cDNA sequence can be used to express in recombinant cells RAFT1 proteins or portions of the RAFT1 protein. Similarly, the cDNA sequence can be used to construct fused genes which will express fusion proteins comprising all or part of the RAFT1 sequence. Having provided the art with the amino acid sequence of the RAFT1 protein, other coding sequences can be devised which differ from that shown in SEQ ID NO:1 by virtue of the degeneracy of the genetic code. Such nucleotide sequences are within the scope of the present invention.

RAFT1 has an apparent molecular weight on SDS polyacrylamide gels of 245 kDa. RAFT2 has an apparent molecular weight on SDS polyacrylamide gels of 35 kDa. Isolated and purified RAFT1 protein can be obtained by means of recombinant DNA technology or by isolating and purifying the protein directly from natural sources. One means of purifying RAFTs involves contacting a preparation of mammalian proteins with FKBP12 in the presence of rapamycin. Those proteins which bind to FKBP12 in the presence of rapamycin can then be separated from those which do not bind. Bound proteins can then be dissociated to yield a preparation of a RAFT protein. It is convenient if the FKBP12 is immobilized, for example, on a solid support. One convenient means is to immobilize FKBP12 on a column-packing matrix. For example, an FKPB12-glutathione-S-transferase fusion protein can be readily bound to glutathione-agarose to provide immobilized FKBP12. Another means of purifying RAFT proteins is by use of a heparin chromatography column. The RAFT proteins bind to the heparin and can be eluted at 300 to 450 mM KCl.

Because of the role of rapamycin in immunological, cell cycle, and neurological functions, it may be desirable to inhibit the expression of RAFT1. One means to accomplish this is to use antisense polynucleotides. Antisense polynucleotides can be made synthetically, according to the sequence provided in SEQ ID NO:1. Alternatively, expression constructs may be used which comprise a promoter operably linked to at least 20 nucloetides of the antisense strand of RAFT1 cDNA. The expression construct directs the synthesis in a cell of an RNA molecule which is complementary to RAFT1 mRNA. Any suitable promoter can be used, depending on the cell system in which expression of the antisense molecule is desired.

The nucleotide sequence of SEQ ID NO:1 can be used to generate probes which comprise at least 15–20 nucleotides of the recited sequence. In some cases probes of 25, 30, 35, 40, 50, or 100 nucleotides may be desired. These probes can be used to screen a library of mammalian DNA molecules. Techniques for making nucleotide probes and screening genomic or cDNA libraries are well known in the art. Alternatively, other RAFT nucleotide sequences can be obtained by amplification of mammalian DNA using as primers one or two polynucleotides comprising at least 10 contiguous nucleotides selected from the sequence shown in SEQ ID NO:1. Techniques for amplification of DNA are also well known in the art.

RAFT1 and RAFT2 can be used to screen substances for potential as therapeutic agents for immunological, cell cycle, and neurological disease states. As described here, rapamycin, FKBP12, RAFT1, and RAFT2 bind to each other and form a complex. Test compounds can be screened for potential therapeutic utility by contacting a test compound with three components: (a) FKBP12; (b) rapamycin; and (c) a protein selected from the group consisting of RAFT1 and RAFT2. The amount of one of the components in the complex is determined, in the presence and in the absence of the substance to be tested. A substance which increases or decreasees the amount of the component in the comlex is a potential therapeutic agent. Means used for determining amounts of components can be any known in the art, including the use of radioactive components, antibodies specific for components, densitometry, etc.

EXAMPLES

The following materials were used in the examples described below. Frozen rat brains stripped of the meninges were obtained from Harlan Bioproducts (Indianapolis, Ind.). Other materials were purchased from the following sources: [γ-^{32}P]-ATP (NEG-02z) from New England Nuclear (Cambridge, Mass.), glutathione-agarose, heart muscle kinase (PKA, #P2645), and heparin-agarose from Sigma Chemical (St. Louis, Mo.), thrombin and antithrombin from Boehringer Mannheim (Indianapolis, Ind.), and disuccinimidyl suberate (DSS) from Pierce (Rockford, Ill.). Rapamycin was a gift of the Wyeth-Ayerst company (Philadelphia, Pa.) and FK506 a gift of the Fujisawa company (Tsukuba City, Japan).

Example 1

Rapamycin Promotes the Binding of FKBP12 to Two Cytosolic Proteins of Mr 245 and 35 kDa

A ^{32}P-radiolabeled FKBP12 probe was used to detect proteins that associate with the immunophilin in the presence of ligand, and are crosslinked to it by the bivalent reagent DSS. The probe was prepared by phosphorylating with [γ^{32}P]ATP a recombinant rat FKBP12 to which two consensus sites for cyclic AMP-dependent protein kinase (PKA) were added at the N-terminus (Blanar and Rutter, 1992; Li et al., 1992). Since this modification did not alter the capacity of the protein to associate with calcineurin in the presence of FK506, the probe can be used to identify a target of the FKBP12-rapamycin complex.

PC12 pheochromocytoma cell cytosolic extracts were incubated with ^{32}P-FKBP12 in the presence or absence of rapamycin and then treated with the crosslinker DSS before gel electrophoretic analysis followed by autoradiography. The drug caused the formation of two protein complexes with radioactive FKBP12, corresponding to bands of Mr 260 and 50 kDa (FIG. 5). Taking into account the 15 kDa Mr of the modified FKBP12 probe, the crosslinked proteins were estimated to be 245 kDa and 35 kDa, respectively. The crosslinked complexes were observed over a wide rapamycin concentration range, but were more prominent at the low concentrations of 1 and 10 nM, possibly because of an inhibitory effect on the association of the higher amounts of ethanol (the solvent of the drug) present at the higher drug concentrations (FIG. 5). Rapamycin also induced the formation of similar complexes when the probe was incubated with cytosolic extracts from several rat tissues, including

liver, kidney, heart, small intestine, thymus, testes, spleen and brain, but no significant differences in abundance of the crosslinked proteins between the tissues were observed. For convenience, further experiments were carried out with whole brain extracts.

The formation of the rapamycin-dependent complexes was specific for FKBP12, since in similar experiments with the related immunophilin ^{32}P-FKBP25, no ligand induced complexes were observed.

PC12 cells were maintained in culture as described (Altin et al., 1991). PC12 cells were lysed in homogenization buffer with 0.3% NP-40 instead of CHAPS. Lysis was accomplished in 2 ml buffer/T-150 flask by repeated vortexing at 4° C. Cell debris was sedimented by centrifugation for 10,000×g for 10 minutes at 4° C.

The labeled, cleaved FKBP12 was diluted to 10,000 cpm/ml in 50 mM Hepes pH 7.5, 1 mg/ml BSA. 10 μl of labeled protein (100,000 cpm total), 10 μl of tissue or PC12 cell extract, and 10 μl of drug dilutant buffer (20 mM Hepes 6.8, 100 mM KCl, 1 mM EGTA, 1 mM DTT) containing either 3-fold the desired final concentration of rapamycin, FK506, or equivalent amounts of ethanol, were mixed and incubated for 1 hour at 4° C. After this incubation, 1 ml of 5.5 mg/ml disuccinimidyl suberate (DSS) was added and the incubation continued for 40 minutes. The reaction was terminated by adding one column volume of 2× concentrated sample buffer (Laemmlli, 1970) containing 50 mM Tris pH 7.4 and processed by SDS-PAGE (10%, unless otherwise specified) and autoradiography.

Example 2

Specificity of the Rapamycin Induced Association: the Interaction of ^{32}P-FKBP12-Rapamycin with the 245 and 35 kDa Proteins is Competed by FK506 and by Unlabeled FKBP12

To investigate further the specificity of the interaction of ^{32}P-FKBP12-rapamycin with the cytosolic proteins, we performed a partial purification to remove endogenous FKBP12, which is present in brain at high concentrations (Steiner et al., 1992). This was accomplished by chromatography on a heparin column, to which the cytosolic proteins that interact with FKBP12-rapamycin bound and could be eluted at 300 to 450 mM KCl (FIG. 1). Free FKBP12, on the other hand, was recovered in the flow-through of this column, as demonstrated by binding to [^{3}H]FK506 (data not shown).

The rat brain extract was applied to a heparin column (2 ml of packed heparin-agarose per brain) at a flow rate of 1.5 ml/min. The column was washed with 10 column volumes of buffer (20 mM Hepes pH 6.8, 200 mM KCl, 1 mM EGTA, 50 mM NaF, 1.5 mM Na_3VO_4, 4 mM DTT, 1 mM PMSF) and the same protease inhibitors as in the homogenization buffer. The material bound to the column was eluted with a linear KCl gradient from 200 to 600 mM in homogenization buffer. Aliquots (10 μl) of the fractions collected were tested in the crosslinking assay and positive fractions were pooled and concentrated in a centriprep-100 (Amicon, Beverly, Mass.) to ⅓ starting volume. The flowthrough of the heparin column was assayed for the presence of FKBP with a ^{3}H-FK506 binding assay, as described (Steiner et al, 1992).

FK506 antagonizes actions of rapamycin, and both drugs compete for the same binding site on FKBP12 (Bierer et al., 1990a; Dumont et al., 1990a). Accordingly, we examined the influence of FK506 on the rapamycin-induced interaction of ^{32}P-FKBP12 with its putative cytosolic targets. At concentrations ranging from 1 nM to 1 μM rapamycin induced the appearance of intense bands representing crosslinked proteins and, at all rapamycin concentrations tested, this effect was antagonized by 1 μM FK506 (FIG. 2A). As expected for ligands of similar affinity for FKBP12, when equal concentrations (1 μM) of rapamycin and FK506 were present, the intensities of the crosslinked bands were reduced by approximately 50% and the reduction progressively increased with increasing ratios of FK506/rapamycin. The heparin column eluate apparently contains limiting amounts of the putative targets of the FKBP12-rapamycin complex, since excess unlabeled FKBP12 (1 μM) completely suppressed the appearance of the crosslinked bands containing labeled FKBP12 (FIG. 2A).

Control experiments (FIG. 2B) confirmed the specificity of the rapamycin effect since the formation of the complex was not induced by several concentrations of FK506 or by ethanol, the solvent of the drugs. These experiments demonstrate that the crosslinked proteins are specific targets of the FKBP12-rapamycin complex and not of the FKBP12-FK506 complex, nor of FKBP12 alone. Therefore, we designate the crosslinked proteins RAFT1 (245 kDa) and RAFT2 (35 kDa) for Rapamycin And FKBP12 Target.

We attempted to separate RAFT1 and RAFT2 under nondenaturing conditions by several chromatography and gel filtration procedures, including DEAE and CM cellulose, reactive dye green 5, and Superose 6 (data not shown). All of these efforts failed, suggesting that RAFT1 and RAFT2 are part of a complex, although it is possible that RAFT2 is a proteolytic fragment of RAFT1 that contains the FKBP12-rapamycin binding site and remains tightly bound to the rest of the polypeptide.

Example 3

Purification of RAFT1

We purified RAFT1 from the heparin column eluate based on its affinity for FKBP12-rapamycin. We constructed a glutathione-S-transferase-FKBP12 fusion protein by cloning, in frame downstream of GST, a cDNA encoding FKBP12 with two N-terminal PKA consensus sites (Smith and Johnson, 1988; Blanar and Rutter, 1992; Li et al., 1992). The encoded protein was expressed in bacteria, purified and immobilized on glutathione-agarose beads. SDS-PAGE analysis of the beads recovered after incubating them with the heparin eluate in the presence or absence of rapamycin shows that the drug induces the binding to the beads of a protein of 245 kDa (FIG. 3). With this simple purification scheme we were able to purify about 5 μg of RAFT1. A low transfer efficiency to nitrocellulose membrane resulted in only 2.5 μg being available for protein sequencing, which corresponds to 10 picomoles of a protein of this size.

Standard techniques of molecular biology cloning were used as described (Sambrook et al., 1989) for the preparation of GST-$(PKA)_2$-FKBP12 and GST-$(PKA)_2$-FKBP25 fusion proteins, unless otherwise specified. All cDNAs obtained with the polymerase chain reaction were sequenced using the Sequenase kit (Amersham, Arlington Heights, Ill.). cDNAs for the rat FKBP12 and FKBP25 were obtained with the PCR using 5' and 3' primers to the corresponding human FKBP12 (Standaert et al., 1990) or FKBP25 (Jin et al., 1992) sequences. The cDNAs were cloned into pBluescript (Stratagene, La Jolla, Calif.).

A 5' primer (PKA-12-1 or PKA-25-1) encoding a BamHI site, two consensus PKA phosphorylation sites (Blanar and Rutter, 1992; Li et al., 1992), and the first 6 amino acids of

FKBP12 or FKBP25 was used with a 3' primer (PKA-12-2 or PKA-25-2) encoding an EcoRI site and the last 6 codons of FKBP12 or FKBP25 in a PCR with Vent Polymerase (New England Biolabs, Beverly, Mass.) using the rat FKBP cDNAs cloned in pBluescript as templates. The amplified DNA fragments were gel purified, digested with BamH1 and EcoR1 and cloned into the pGEX-2T vector (Pharmacia, Upsala, Sweden) that had been linearized with the same restriction enzymes. The resulting construct was used to transform BL21 (DE3) *E. coli* (Novagen, Madison, Wis.) in which expression can be induced with IPTG.

The primer sequences were as follows:

PKA-12-1: 5' CCGGATCCCGTCGAGCTTCAGTTGAACTACGGCGTGC TTCTGTAGCCATGGGAGTGCAGGTGGA 3' (SEQ ID NO:5)

PKA-12-2: 5' GGCCGGAATTCTCATTCCAGTTTTAGAA 3' (SEQ ID NO:6)

PKA-25-1: 5' CCGGATCCCGTCGAGCTTCAGTTGAACTACGGCGTGC TTCTGTAGCCATGGCGGCGGCCGTTCC 3' (SEQ ID NO:11)

PKA-25-2: 5' GGCCGGAATTCTCAATCAATATCCACTA 3' (SEQ ID NO:12)

The fusion proteins were purified with glutathione-agarose as previously described (Smith and Johnson, 1988) from bacterial cultures induced with 1 mM IPTG.

The concentrated heparin column eluate was incubated for 2 hours at 4° C. with 1/50 volume of glutathione-agarose to remove endogenous glutathione binding proteins. The beads were removed by centrifugation at 1000×g for 3 minutes. Fresh glutathione-agarose (1/500 volume) and 20 μg of purified GST-PKA-FKBP12 fusion protein were then added to the cleared heparin column eluate with or without 100 nM rapamycin. After a 1 hour incubation at 4° C., the bead were washed 5× with 1.5 ml ice-cold PBS containing 1% Triton X-100 and 500 mM NaCl. The beads were transferred to 3× volume SDS-PAGE sample buffer, and the eluted proteins fractionated by SDS-PAGE and the gel silver stained.

Whether RAFT2 was also bound to the beads could not be determined in this experiment, because its presence would be masked by the large band of similar Mr corresponding to the GST-(PKA)$_2$-FKBP12 fusion protein. When smaller fusion proteins, such as an epitope-tagged FKBP12, were employed for the affinity matrix, the binding of the 35 kDa RAFT2 could also be observed.

The immunophilin fusion proteins containing N-terminal phosphorylation sites for PKA were labeled with a modification of published procedures (Blanar et. al., 1992, Li et. al., 1992). 10 ng of purified GST-PKA-FKBP12 or 25 was mixed with 40 units of PKA and 100 mCi of [γ-P^{32}]-ATP in a buffer containing 20 mM Hepes pH 7.7, 100 mM NaCl, 12 mM $MgCl_2$, 1 mM DTT.

After a 1.5 hour at 37° C. the incubation mixture containing labeled fusion protein was dialyzed twice against 1L of thrombin cleavage buffer (50 mM Tris pH 7.4, 150 mM NaCl, 2.5 mM $CaCl_2$). The labeled fusion protein was cleaved by adding an equal volume of thrombin cleavage buffer containing 2 mg/ml thrombin and incubating at room temperature for 2 hours. The thrombin was inactivated by adding an equal volume of a stop solution consisting of 1 mM DTT, 1 mM PMSF, 100 units/ml antithrombin III. The specific activity of the probes was estimated at 1×10^5 cpm/pmol of the protein.

Example 4

Protein Sequencing of RAFT1: Homology to TOR1 and TOR2

Affinity purified RAFT1 was separated by SDS-polyacrylamide gel electrophoresis from other proteins that adsorbed to the glutathione-agarose beads, transferred to nitrocellulose membrane, and digested with trypsin. Fractionation of the tryptic digest by narrow-bore reverse phase chromatography yielded a complex pattern of over a hundred peaks whose purity was assessed by mass spectroscopy. In most cases, the peaks exhibited multiple mass to charge peak values and it was necessary to rechromatograph these peak fractions on a microbore columns of different selectivity.

For protein sequence analysis affinity purified material derived from 50 brains was fractionated by SDS-PAGE and transferred to nitrocellulose membranes. The proteins transferred were visualized by Ponceau S staining, the 245 kDa RAFT1 band excised and processed for internal amino acid sequence analysis, essentially as described (Tempst et al., 1990).

Membrane-bound protein, about 2.5 μg, was subjected to in-situ proteolytic cleavage using 1 μg trypsin (Sequencing Grade; Boehringer-Mannheim) in 25 ml 100 mM NH_4HCO_3 (supplemented with 10% acetonitrile and 3% Tween-80) at 37° C. for 3 hours. The resulting peptide mixture was reduced and S-alkylated with, respectively, 0.1% β-mercapto ethanol and 0.3% 4-vinyl pyridine, and fractionated by two-dimensional reversed phase HPLC.

For the primary separations, a 2.1 mm Vydac C4 (214TP54) column was used with gradient elution at a flow rate of 100 μl/min. HPLC solvents and system configuration were as described (Tempst et al., 1990), with improved dead volume reduction through the use of glass capillary tubing (Elicone and Tempst, unpublished). Identification of Trp-containing peptides was done by manual ratio analysis of absorbances at 297 and 277 nm, monitored in real time using an Applied Biosystems model 1000S diode-array detector (Tempst et al., 1990). Fractions were collected by hand, kept on ice for the duration of the run and then stored at −70° C. before repurification and/or analysis. An enzyme blank was done on an equally sized strip of nitrocellulose cut from a blank area of the same blot. Repurifications (second dimension LC) were carried out on a 1.0 mm SGE ODS-2 C18 column using the same solvent system but at a flow rate of 30 μl/min. (C. Elicone, M. Lui, S. Geromanos, H. Erdjument-Bromage, P. Tempst, in press). Samples were always acidified (20% TFA final concentration) and then diluted twofold with 0.1% TFA before rechromatography.

Sequences of 23 peptides separated in this fashion were determined by a combination of automatic Edman degradation, matrix-assisted laser desorption mass-spectroscopy, and UV spectroscopy.

Peak fractions over background were analyzed by a combination of automated Edman degradation and matrix-assisted laser-desorption (MALDI-TOF) mass spectrometry (Geromanos et al., 1994; Elicone et al., 1994). After storage, column fractions were supplemented with neat TFA (to give a final concentration of 10%) before loading onto the sequencer disc and mass spectrometer probe tips. Peptide mass analysis (on 2% aliquots) was carried out using a model LaserTec ResearcH MALDI-TOF instrument (Vestec), with a 337 nm output nitrogen laser and 1.2 m flight tube. The matrix was a-cyano-4-hydroxy cinnamic acid, and a 28 kV ion acceleration and 4.3 kV multiplier voltage were used. Laser power and number of acquisitions were adjusted as judged from optimal deflections of specific maxima, using a Tektronix TDS 520 digitizing oscilloscope. M/z (mass to charge) spectra were generated from the time-of-flight files using GRAMS data analysis software. Every sample was analyzed twice, in the presence and

absence of a calibrant (25 femtomoles APID), as described (Geromanos et al., 1994). Chemical sequencing (on 95% of the sample) was done using a model 477A instrument from Applied Biosystems (AB). Stepwise liberated PTH-amino acids were identified using an "on-line" 120A HPLC system (AB) equipped with a PTH C18 (2.1×220 mm; 5 micron particle size) column (AB). Instruments and procedures were optimized for femtomole level phenylthiohydantoin amino acid analysis as described (Tempst and Riviere, 1990; Erdjument-Bromage et al., 1993).

Peptide average isotopic masses were summed from the identified residues (including the presumed ones) using ProComp version 1.2 software (obtained from Dr. P. C. Andrews, University of Michigan, Ann Arbor, Mich.). Peptide sequences were compared to entries in various sequence databases using the National Center for Biotechnology Information (NCBI) BLAST program (Altschul et al. 1990). Lower stringency alignments between all peptides and selected proteins were done using the Lipman-Pearson algorithm, available in the 'Lasergene' software package (DNASTAR).

Several protein sequence databases (PIR, SwissProt, translated Genbank) were searched for sequences that match any of the 23 peptide sequences obtained from microsequencing of RAFT1. While sequence similarities with hundreds of different proteins were obtained for many of the 23 peptides, none perfectly matched with any of the entries in the databases, nor did any protein match more than one or two peptides, other than the yeast proteins TOR1 and TOR2 (Kunz et al., 1993). Strikingly, sixteen out of the 23 peptides of RAFT1 could be aligned with the yeast TOR sequences, with varying degrees of similarity (FIG. 4).

Example 5

Molecular Cloning of RAFT1

To generate a probe for isolating a RAFT1 cDNA two degenerate oligonucleotides were used in a mixed oligonucleotide polymerase chain reaction (PCR) (Gould et al, 1989) with rat brain cDNA as template. The sense primer was made to a peptide sequence (TYDPNQP, SEQ ID NO:7) obtained from microsequencing of RAFT1, while the antisense primer corresponds to a sequence (HIDFGD, SEQ ID NO:8) conserved between TOR1, TOR2, and p110 PI-3 Kinase. From the alignments of the RAFT1 peptides to the TORs, this sequence was expected to be 220 amino acids downstream of that encoded by the sense primer. The predicted 660 bp PCR product was obtained, cloned, and its authenticity was verified by DNA sequencing, which showed that it encoded two other sequenced tryptic peptides. The PCR product was, therefore, used as a probe (3' probe) to screen a rat striatum cDNA library, which yielded a 5.5 kb partial cDNA clone. An antisense oligonucleotide to the extreme 5' end of this cDNA was then used in a PCR reaction with a degenerate sense oligonucleotide to another peptide sequence (NDQVFE, SEQ ID NO:9) obtained from microsequencing. The predicted 1.1 kb PCR product was obtained, cloned and used as probe (5' probe) to screen a rat brainstem cDNA library in parallel with the original 3' probe. Phage plaques that hybridized with both probes were isolated and one was found to carry a 8.6 kb insert. A degenerate sense oligonucleotide corresponding to the amino acid sequence TYDPNQP, which was obtained from microsequencing of RAFT1 and aligns to residues 2086 to 2093 of TOR2, and a degenerate antisense primer corresponding to amino acids 2296 to 2301 (HIDFGD, SEQ ID NO:8) of TOR2 were used in a PCR reaction with rat whole brain cDNA as template. The protocol for the PCR was: an initial 5 min at 94° C., followed by 35 cycles of 94° C. for 40s, 56° C. for 1 min, 72° C. for 1 min, and a final incubation at 72° C. for 5 min. The PCR products were fractionated on a 1.1% agarose gel, the expected 700 bp DNA fragment purified and subcloned into pBluescript.

The RAFT-1 cDNA fragment in pBluescript was amplified by PCR, the product gel purified and labeled by nick translation with a commercial kit (Boehringer Mannheim). This probe (designated 3' probe) was used to screen 1×10^6 phage plaques of a rat striatum λ ZAP library (Stratagene), as described (Sambrook et al.). Forty seven positive clones were identified and 10 of them were purified by an additional two rounds of screening. None of the inserts contained a complete open reading frame. The 5' end of the largest insert (5.5kb) was used to design a 18 bp antisense oligonucleotide (3.1 as) that was used in another PCR reaction with rat whole brain cDNA as template and a degenerate oligonucleotide corresponding to the amino acid sequence NDQVFE (SEQ ID NO:9 part of a peptide obtained from microsequencing) as the sense primer. The PCR products were fractionated on a 1% agarose gel and a DNA fragment of 1.1 kb isolated and cloned into the vector pCR-II using the TA cloning kit (Invitrogen, San Diego, Calif.). The cDNA fragment was amplified by PCR, the product gel purified and labeled by nick translation. This probe (designated 5' probe) was used to screen 1×10^6 phage plaques from a rat brainstem λ ZAP library. Duplicate filters were screened with the 3' probe. Eight clones hybridized with both the 5' and 3' probes, and five of these were purified through 2 additional rounds of screening. One clone contained a 8.6 kb insert that encodes all 23 peptide sequences obtained by microsequencing.

PCR primer sequences were as follows:

TYDPNQP (SEQ ID NO:7): 5'-GGGGGATCCACNTA (C/T) GA(C/T)CCNAA(C/T) CA(A/G)C-3' (SEQ ID NO:13)

HIDFGD (SEQ ID NO:8): 5'-GCGGAATTC(G/A) TCNCC(G/A)AA(G/A)TC(T/G/A) AT(G/A)TG-3' (SEQ ID NO:14)

NDQVFE (SEQ ID NO:9): 5'-GGGGGATCCAA(C/T) GA(C/T)CA(G/A)GTNTT (T/C)GA-3' (SEQ ID NO:15)

3.1as: 5'-GAGCCACCACGATTTGCT-3' (SEQ ID NO:10)

cDNA clones were sequenced using the flourescent terminator method of cycle sequencing on a Applied Biosystems 373a automated DNA sequencer at the DNA analysis Facility of the Johns Hopkins University (Smith et al., 1986; McCombie et al, 1992), or with the dideoxy chain termination method using the Sequenase kit (Amersham, Arlington Heights, Ill.). Oligonucleotides used for sequencing were synthesized on an ABI 394 synthesizer following ABI protocols. DNA sequence data was analyzed using Sequencher software from Gene Codes (Ann Arbor, Mich.). Protein alignments were done with help from the e-mail service of the Computational Biochemistry Research Group (CBRG) at the ETH.

This cDNA contains an open reading frame of 7.6 kb with an initiation methionine codon that conforms to the Kozak consensus sequence (Kozak, 1986) and is preceded by an in-frame termination codon. The protein encoded by this open reading frame contains all 23 peptide sequences obtained by microsequencing of RAFT1 (FIG. 4). Interestingly, none of the peptides sequenced correspond to the C-terminal 250 amino acids of RAFT1, which may indicate that this portion of the protein was proteolytically removed during the purification.

The RAFT1 cDNA predicts a protein of 2550 amino acids with a molecular mass of 289 kDa and a pI of 6.8. Over its entire sequence RAFT1 is 43% identical to TOR2 and 39% identical to TOR1 (FIG. 4). The C-terminal 600 amino acids of RAFT1, which, by analogy to the TORs (Cafferkey et al., 1993; Kunz et al., 1993; Helliwell et al., 1994), is predicted to contain lipid kinase activities, is 65% identical to the yeast proteins. The RAFT1 protein has over 20 consensus sites for phosphorylation by protein kinase C (PKC), including one at $serine_{2035}$, which is in the analogous position to the serine (S_{1972} in TOR1 and S_{1975} in TOR2) found mutated to arginine in rapamycin resistant yeast (boxed residues in FIG. 4).

The predicted RAFT1 protein is 80 amino acids longer than the TOR proteins, and contains several regions with no apparent homology to the yeast proteins, the largest being a 93 amino acid insertion corresponding to residues 270 to 363 of RAFT1. It is possible that these regions are generated by alternative splicing of exons that may be tissue specific to the brain. They are unlikely to be the translation product of unspliced introns because they were found in several cDNA clones isolated from different libraries and the DNA sequence does not reveal consensus splice junction sites.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 15

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8598 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus
(G) CELL TYPE: pheochromocytoma
(H) CELL LINE: PC12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GAGCGTCTGA GAGAGGAGAT GGAGGAAATC ACCCAGCAGC AGCTGGTACA TGACAAGTAC     960

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AAGTGGACTC TGGTTAATGA CGAGACCCAG GCTAAGATGG CCCGGATGGC TGCTGCAGCA 4620

GCATGGGGTT TAGGTCAGTG GGACAGCATG GAGGAGTACA CCTGTATGAT TCCTCGGGAT 4680

ACTCACGATG GAGCATTCTA CAGAGCAGTG TTGGCACTGC ATCAGGATCT CTTCTCCTTG 4740

GCTCAACAGT GCATTGACAA GGCCAGGGAC CTGCTGGACG CCGAGCTGAC TGCCATGGCA 4800

GGGGAGAGCT ACAGCCGAGC CTATGGGGCC ATGGTTTCTT GCCACATGCT GTCCGAGCTG 4860

GAGGAGGTTA TCCAGTACAA ACTCGTCCCG GAGCGACGGG AGATCATCCG CCAGATCTGG 4920

TGGGAGAGAC TGCAGGGCTG CCAGCGTATT GTAGAGGACT GGCAGAAAAT CCTCATGGTC 4980

CGGTCCCTTG TGGTCAGCCC TCACGAGGAC ATGAGAACCT GGCTCAAGTA CGCAAGCCTG 5040

TGTGGCAAGA GCGGCAGACT GGCTCTTGCT CATAAAACCT TAGTGTTGCT CTTGGGAGTT 5100

GATCCATCTC GGCAACTTGA CCATCCTCTG CCAACAGTTC ACCCTCAAGT GACCTATGCC 5160

TACATGAAAA ACATGTGGAA AAGCGCTCGG AAGATTGATG CCTTCCAGCA CATGCAGCAC 5220

TTTGTGCAGA CCATGCAGCA GCAGGCCCAG CACGCCATTG CCACAGAGGA CCAGCAGCAC 5280

AAGCAGGAGC TGCATAAGCT CATGGCCAGG TGTTTTCTGA AACTTGGGGA GTGGCAGCTG 5340

AACCTCCAGG GCATCAACGA GAGCACCATC CCCAAGGTGC TACAGTACTA CAGTGCTGCC 5400

ACAGAGCATG ACCGCAGCTG GTATAAGGCT TGGCACGCAT GGGCAGTGAT GAACTTTGAA 5460

GCCGTGCTAC ACTACAAACA TCAGAACCAA GCCCGCGATG AGAAGAAGAA ACTGCGCCAT 5520

GCCAGCGGGG CCAACATCAC CAATGCCACC ACCACTGCCA CCACCGCTGC CTCCGCTGCC 5580

GCTGCCACCA GCACAGAGGG CAGCAACAGT GAAAGTGAAG CCGAGAGCAA TGAGAGCAGC 5640

CCCACCCCGT CCCCTCTGCA GAAGAAGGTC ACTGAGGATT TGTCCAAAAC CCTCTTGTTG 5700

TACACTGTCC CTGCTGTCCA AGGCTTCTTC CGTTCTATCT CCTTGTCGAG AGGCAACAAC 5760

CTCCAGGATA CACTCAGAGT CCTCACCTTG TGGTTTGATT ATGGTCACTG GCCAGATGTC 5820

AATGAAGCCC TGGTGGAAGG GGTGAAGGCC ATACAGATTG ACACTTGGTT ACAGGTTATA 5880

CCTCAGCTCA TTGCAAGAAT TGACACGCCC AGACCCTTGG TGGGCCGGCT CATTCACCAG 5940

CTCCTCACAG ATATTGGTCG GTACCACCCA CAGGCCCTCA TCTACCCCCT GACGGTGGCT 6000

TCTAAGTCTA CCACCACAGC CCGTCACAAT GCAGCCAACA AGATCCTGAA GAACATGTGC 6060

GAGCACAGCA ACACGCTAGT ACAGCAGGCC ATGATGGTGA GTGAAGAGCT GATTCGAGTA 6120

GCCATCCTCT GGCATGAGAT GTGGCATGAA GGCCTAGAAG AGGCCTCTCG CTTGTACTTT 6180

GGGGAGAGGA ACGTCAAAGG CATGTTTGAG GTGCTGGAGC CCTGCATGC TATGATGGAA 6240

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CGCGGTCCCC AGACCCTGAA GGAAACGTCC TTTAATCAGG CATATGGTCG AGATTTAATG   6300

GAGGCACAAG AATGGTGCCG AAAGTACATG AAATCAGGGA ACGTCAAGGA CCTCACCCAA   6360

GCCTGGGACC TCTACTATCA CGTGTTCAGA CGGATCTCCA AGCAGCTACC ACAGCTCACA   6420

TCCCTGGAGC TGCAGTATGT GTCCCCCAAA CTTTTGATGT GCAGAGACCT TGAATTGGCT   6480

GTGCCAGGAA CATATGACCC CAACCAGACA ATCATTCGCA TTCAGTCCAT AGCCCCGTCT   6540

TTGCAAGTCA TCACATCCAA GCAGAGGCCT CGGAAGCTGA CCCTGATGGG CAGCAATGGG   6600

CACGAGTTTG TTTTCCTCCT GAAAGGCCAT GAAGATCTGC GGCAGGACGA GCGAGTGATG   6660

CAGCTCTTTG GCCTGGTGAA CACACTCCTA GCCAATGACC CAACTTCTCT TCGAAAGAAC   6720

CTCAGCATCC AGAGATACGC CGTCATTCCT CTGTCCACCA ACTCGGGCCT GATTGGCTGG   6780

GTGCCCCACT GTGACACGCT GCATGCCCTC ATCCGGGACT ACAGAGAGAA GAAGAAGATC   6840

CTGCTGAACA TCGAGCACCG CATCATGCTG CGGATGGCTC CTGACTATGA CCACCTGACT   6900

CTGATGCAGA AGGTGGAGGT GTTTGAGCAT GCTGTCAACA ACACAGCCGG GGATGACCTG   6960

GCCAAGCTGC TGTGGCTGAA AAGCCCCAGC TCAGAGGTGT GGTTTGACCG AAGAACCAAT   7020

TATACTCGCT CCCTGGCTGT CATGTCCATG GTTGGATACA TTTTAGGCCT TGGAGACAGG   7080

CACCCATCCA ACCTGATGCT GGACCGGCTG AGTGGAAAGA TCCTGCACAT TGACTTTGGG   7140

GACTGCTTTG AGGTTGCTAT GACCAGAGAG AAATTTCCAG AAAAGATTCC ATTTAGACTA   7200

ACAAGAATGT TGACCAATGC TATGGAGGTT ACCGGTCTCG ATCGCAACTA TAGAACCACA   7260

TGCCACACAG TGATGGAGGT GCTTCGGGAG CACAAGGACA GTGTCATGGC TGTGCTAGAA   7320

GCCTTTGTCT ATGACCCTCT GCTGAATTGG AGGCTGATGG ACACAAATGC CAAAGGCAAC   7380

AAGCGGTCCC GAACCAGGAC AGACTCCTAT TCTGCAGGCC AGTCAGTAGA AATTTTGGAC   7440

GGTGTAGAAC TTGGAGAACC AGCCCATAAG AAAACAGGGA CCACTGTGCC AGAATCCATC   7500

CATTCTTTCA TTGGAGATGG TTTGGTGAAA CCAGAAGCCT TAAACAAGAA AGCTATTCAG   7560

ATTATTAACA GGGTTCGAGA TAAGCTCACT GGTCGGGATT TCTCTCATGA TGACACTTTG   7620

GATGTTCCAA CCCAAGTGGA ACTGCTTATC AAGCAAGCGA CATCTCATGA GAACCTCTGC   7680

CAGTGCTACA TTGGCTGGTG TCCTTTCTGG TAACCAAGGC CTGGCAAAGA AAATCATCTC   7740

CTCCGATGCT TTTGTACCTT GGTCTGTGCT TCCAGTGGAC TGAAACCATG GTCATAAAGT   7800

TGGACTTTGT TAATATTTTG AAATGTATAT GAAAAGAACT ACTGTATATT CAAAGTTGGC   7860

TTATGCCAAC CTCCTAGCTG CTGTTGAAAA GACACTGTCA GAAACACAAG GCTTGATTCA   7920

GTTCCCAGGA CAGTGAAACA CAGTAATCCT ACAGAAACCA AGCCTTTGAT TTTGGGAGAA   7980

CAGAAGATGA GTAACTGACT AAGAAATACG GGTTTGGACT TAACTTACAG AAGAACTCAT   8040

CATACGCATT TGCTGACCGA ATAATCTAGT TGATCCTCTC AACCAGGGGC TTCAACAGCA   8100

AGGACACAGA TGTCAGCACT CCACCATCCT GTTACCTCAC CCGTCCCTGG ATGCAGTGGC   8160

AACATCTGCA GGATGGGCCA CCGTGTGTGT AAGAAGATCT GTCTTCCACC TGATCCCATG   8220

ATGCTGAACC TCACAGAGCC GGCCTTCCAG GAAGGACGTT TGCTCAGACG CCTGGCCACC   8280

GAGGATGAGC AGGTGTGCCA GGATCTCAGT GCAGGGTCCA CGCTGGCCCT GCTGCTGTGT   8340

TCAGTGAGGG ATGGATATGT TGTGTTTGCA GCAGGGACTC AGAACACAAA TGCTTTTGTG   8400

GAAGTGCTGA TCTCAGAGGG ACACTAGCGC AGGTTGTGAA TTAAGAGCAA AGTAAATATC   8460

CAACTAAACA CAAAGTATAA GTGAAGCCAC ATCTAGACAC CATTGTATCT GAGTAATTTT   8520

TGTGCCAATA AATGACATCA GAATTTTAAA AGTAAAAAAA ACGATATCAA GCTTATCGAT   8580

ACCGTCGACC TCGAGGGG                                                 8598
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2549 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus rattus
(F) TISSUE TYPE: pheochromocytoma
(G) CELL TYPE: PC12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Leu Gly Thr Gly Pro Ala Thr Ala Thr Ala Gly Ala Ala Thr Ser
1               5                   10                  15

Ser Asn Val Ser Val Leu Gln Gln Phe Ala Ser Gly Leu Lys Ser Arg
            20                  25                  30

Asn Glu Glu Thr Arg Ala Lys Ala Ala Lys Glu Leu Gln His Tyr Val
        35                  40                  45

Thr Met Glu Leu Arg Glu Met Ser Gln Glu Glu Ser Thr Arg Phe Tyr
    50                  55                  60

Asp Gln Leu Asn His His Ile Phe Glu Leu Val Ser Ser Ser Asp Ala
65                  70                  75                  80

Asn Glu Arg Lys Gly Gly Ile Leu Ala Ile Ala Ser Leu Ile Gly Val
                85                  90                  95

Glu Gly Gly Asn Ser Thr Arg Ile Gly Arg Phe Ala Asn Tyr Leu Arg
            100                 105                 110

Asn Leu Leu Pro Ser Ser Asp Pro Val Val Met Glu Met Ala Ser Lys
        115                 120                 125

Ala Ile Gly Arg Leu Ala Met Ala Gly Asp Thr Phe Thr Ala Glu Tyr
    130                 135                 140

Val Glu Phe Glu Val Lys Arg Ala Leu Glu Trp Leu Gly Ala Asp Arg
145                 150                 155                 160

Asn Glu Gly Arg Arg His Ala Ala Val Leu Val Leu Arg Glu Leu Ala
                165                 170                 175

Ile Ser Val Pro Thr Phe Phe Phe Gln Gln Val Gln Pro Phe Phe Asp
            180                 185                 190

Asn Ile Phe Val Ala Val Trp Asp Pro Lys Gln Ala Ile Arg Glu Gly
        195                 200                 205

Ala Val Ala Ala Leu Arg Ala Cys Leu Ile Leu Thr Thr Gln Arg Glu
    210                 215                 220

Pro Lys Glu Met Gln Lys Pro Gln Trp Tyr Arg His Thr Phe Glu Glu
225                 230                 235                 240

Ala Glu Lys Gly Phe Asp Glu Thr Leu Ala Lys Glu Lys Gly Met Asn
                245                 250                 255

Arg Asp Asp Arg Ile His Gly Ala Leu Leu Ile Leu Asn Glu Leu Val
            260                 265                 270

Arg Ile Ser Ser Met Glu Gly Glu Arg Leu Arg Glu Glu Met Glu Glu
        275                 280                 285

Ile Thr Gln Gln Gln Leu Val His Asp Lys Tyr Cys Lys Asp Leu Met
    290                 295                 300
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Gly Phe Gly Thr Lys Pro Arg His Ile Thr Pro Phe Thr Ser Phe Gln
305                 310                 315                 320

Ala Val Gln Pro Gln Gln Ser Asn Ala Leu Val Gly Leu Leu Gly Tyr
                325                 330                 335

Ser Ser His Gln Gly Leu Met Gly Phe Gly Ala Ser Pro Ser Pro Thr
            340                 345                 350

Lys Ser Thr Leu Val Glu Ser Arg Cys Cys Arg Asp Leu Met Glu Glu
        355                 360                 365

Lys Phe Asp Gln Val Cys Gln Trp Val Leu Lys Cys Arg Ser Ser Lys
    370                 375                 380

Asn Ser Leu Ile Gln Met Thr Ile Leu Asn Leu Leu Pro Arg Leu Val
385                 390                 395                 400

Ala Phe Arg Pro Ser Ala Phe Thr Asp Thr Gln Tyr Leu Gln Asp Thr
                405                 410                 415

Met Asn His Val Leu Ser Cys Val Lys Lys Glu Lys Glu Arg Thr Ala
            420                 425                 430

Ala Phe Gln Ala Leu Gly Leu Leu Ser Val Ala Val Arg Ser Glu Phe
        435                 440                 445

Lys Val Tyr Leu Pro Arg Val Leu Asp Ile Ile Arg Ala Ala Leu Pro
    450                 455                 460

Pro Lys Asp Phe Ala His Lys Arg Gln Lys Thr Val Gln Val Asp Ala
465                 470                 475                 480

Thr Val Phe Thr Cys Ile Ser Met Leu Ala Arg Ala Met Gly Pro Gly
                485                 490                 495

Ile Gln Gln Asp Ile Lys Glu Leu Leu Glu Pro Met Leu Ala Val Gly
            500                 505                 510

Leu Ser Pro Ala Leu Thr Ala Val Leu Tyr Asp Leu Ser Arg Gln Ile
        515                 520                 525

Pro Gln Leu Lys Lys Asp Ile Gln Asp Gly Leu Leu Lys Met Leu Ser
    530                 535                 540

Leu Val Leu Met His Lys Pro Leu Arg His Pro Gly Met Pro Lys Gly
545                 550                 555                 560

Leu Ala His Gln Leu Ala Ser Pro Gly Leu Thr Thr Leu Pro Glu Ala
                565                 570                 575

Ser Asp Val Ala Ser Ile Thr Leu Ala Leu Arg Thr Leu Gly Ser Phe
            580                 585                 590

Glu Phe Glu Gly His Ser Leu Thr Gln Phe Val Arg His Cys Ala Asp
        595                 600                 605

His Phe Leu Asn Ser Glu His Lys Glu Ile Arg Met Glu Ala Ala Arg
    610                 615                 620

Thr Cys Ser Arg Leu Leu Thr Pro Ser Ile His Leu Ile Ser Gly His
625                 630                 635                 640

Ala His Val Val Ser Gln Thr Ala Val Gln Val Val Ala Asp Val Leu
                645                 650                 655

Ser Lys Leu Leu Val Val Gly Ile Thr Asp Pro Asp Pro Asp Ile Arg
            660                 665                 670

Tyr Cys Val Leu Ala Ser Leu Asp Glu Arg Phe Asp Ala His Leu Ala
        675                 680                 685

Gln Ala Glu Asn Leu Gln Ala Leu Phe Val Ala Leu Asn Asp Gln Val
    690                 695                 700

Phe Glu Ile Arg Glu Leu Ala Ile Cys Thr Val Gly Arg Leu Ser Ser
705                 710                 715                 720

Met Asn Pro Ala Phe Val Met Pro Phe Leu Arg Lys Met Leu Ile Gln
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                725                 730                 735

Ile Leu Thr Glu Leu Glu His Ser Gly Ile Gly Arg Ile Lys Glu Gln
            740                 745                 750

Ser Ala Arg Met Leu Gly His Leu Val Ser Asn Ala Pro Arg Leu Ile
        755                 760                 765

Arg Pro Tyr Met Glu Pro Ile Leu Lys Ala Leu Ile Leu Lys Leu Lys
    770                 775                 780

Asp Pro Asp Pro Asp Pro Asn Pro Gly Val Ile Asn Asn Val Leu Ala
785                 790                 795                 800

Thr Ile Gly Glu Leu Ala Gln Val Ser Gly Leu Glu Met Arg Lys Trp
                805                 810                 815

Val Asp Glu Leu Phe Val Ile Ile Met Asp Met Leu Gln Asp Ser Ser
            820                 825                 830

Leu Leu Ala Lys Arg Gln Val Ala Leu Trp Thr Leu Gly Gln Leu Val
        835                 840                 845

Ala Ser Thr Gly Tyr Val Val Glu Pro Tyr Arg Lys Tyr Pro Thr Leu
    850                 855                 860

Leu Glu Val Leu Leu Asn Phe Leu Lys Thr Glu Gln Asn Gln Gly Thr
865                 870                 875                 880

Arg Arg Glu Ala Ile Arg Val Leu Gly Leu Leu Gly Ala Leu Asp Pro
                885                 890                 895

Tyr Lys His Lys Val Asn Ile Gly Met Ile Asp Gln Ser Arg Asp Ala
            900                 905                 910

Ser Ala Val Ser Leu Ser Glu Ser Lys Ser Ser Gln Asp Ser Ser Asp
        915                 920                 925

Tyr Ser Thr Ser Glu Met Leu Val Asn Met Gly Asn Leu Pro Leu Asp
    930                 935                 940

Glu Phe Tyr Pro Ala Val Ser Met Val Ala Leu Met Arg Ile Phe Arg
945                 950                 955                 960

Asp Gln Ser Leu Ser His His His Thr Met Val Val Gln Ala Ile Thr
                965                 970                 975

Phe Ile Phe Lys Ser Leu Gly Leu Lys Cys Val Gln Phe Leu Pro Gln
            980                 985                 990

Val Met Pro Thr Phe Leu Asn Val Ile Arg Val Cys Asp Gly Ala Ile
        995                 1000                1005

Arg Glu Phe Leu Phe Gln Gln Leu Gly Met Leu Val Ser Phe Val Lys
    1010                1015                1020

Ser His Ile Arg Pro Tyr Met Asp Glu Ile Val Thr Leu Met Arg Glu
1025                1030                1035                1040

Phe Trp Val Met Asn Thr Ser Ile Gln Ser Thr Ile Ile Leu Leu Ile
                1045                1050                1055

Glu Gln Ile Val Val Ala Leu Gly Gly Glu Phe Lys Leu Tyr Leu Pro
            1060                1065                1070

Gln Leu Ile Pro His Met Leu Arg Val Phe Met His Asp Asn Ser Gln
        1075                1080                1085

Gly Arg Ile Val Ser Ile Lys Leu Leu Ala Ala Ile Gln Leu Phe Gly
    1090                1095                1100

Ala Asn Leu Asp Asp Tyr Leu His Leu Leu Leu Pro Pro Ile Val Lys
1105                1110                1115                1120

Leu Phe Asp Ala Pro Glu Val Pro Leu Pro Ser Arg Lys Ala Ala Leu
                1125                1130                1135

Glu Thr Val Asp Arg Leu Thr Glu Ser Leu Asp Phe Thr Asp Tyr Ala
            1140                1145                1150
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Ser Arg Ile Ile His Pro Ile Val Arg Thr Leu Asp Gln Ser Pro Glu
        1155                1160                1165

Leu Arg Ser Thr Ala Met Asp Thr Leu Ser Ser Leu Val Phe Gln Leu
    1170                1175                1180

Gly Lys Lys Tyr Gln Ile Phe Ile Pro Met Val Asn Lys Val Leu Val
1185                1190                1195                1200

Arg His Arg Ile Asn His Gln Arg Tyr Asp Val Leu Ile Cys Arg Ile
                1205                1210                1215

Val Lys Gly Tyr Thr Leu Ala Asp Glu Glu Glu Asp Pro Leu Ile Tyr
            1220                1225                1230

Gln His Arg Met Leu Arg Ser Ser Gln Gly Asp Ala Leu Ala Ser Gly
        1235                1240                1245

Pro Val Glu Thr Gly Pro Met Lys Lys Leu His Val Ser Thr Ile Asn
    1250                1255                1260

Leu Gln Lys Ala Trp Gly Ala Ala Arg Arg Val Ser Lys Asp Asp Trp
1265                1270                1275                1280

Leu Glu Trp Leu Arg Arg Leu Ser Leu Glu Leu Leu Lys Asp Ser Ser
                1285                1290                1295

Ser Pro Ser Leu Arg Ser Cys Trp Ala Leu Ala Gln Ala Tyr Asn Pro
            1300                1305                1310

Met Ala Arg Asp Leu Phe Asn Ala Ala Phe Val Ser Cys Trp Ser Glu
        1315                1320                1325

Leu Asn Glu Asp Gln Gln Asp Glu Leu Ile Arg Ser Ile Glu Leu Ala
    1330                1335                1340

Leu Thr Ser Gln Asp Ile Ala Glu Val Thr Gln Thr Leu Leu Asn Leu
1345                1350                1355                1360

Ala Glu Phe Met Glu His Ser Asp Lys Gly Pro Leu Pro Leu Arg Asp
                1365                1370                1375

Asp Asn Gly Ile Val Leu Leu Gly Glu Arg Ala Ala Lys Cys Arg Ala
            1380                1385                1390

Tyr Ala Lys Ala Leu His Tyr Lys Glu Leu Glu Phe Gln Lys Gly Pro
        1395                1400                1405

Thr Pro Ala Ile Leu Glu Ser Leu Ile Ser Ile Asn Asn Lys Leu Gln
    1410                1415                1420

Gln Pro Glu Ala Ala Ser Gly Val Leu Glu Tyr Ala Met Lys His Phe
1425                1430                1435                1440

Gly Glu Leu Glu Ile Gln Ala Thr Trp Tyr Glu Lys Leu His Glu Trp
                1445                1450                1455

Glu Asp Ala Leu Val Ala Tyr Asp Lys Lys Met Asp Thr Asn Lys Asp
            1460                1465                1470

Asp Pro Glu Leu Met Leu Gly Arg Met Arg Cys Leu Glu Ala Leu Gly
        1475                1480                1485

Glu Trp Gly Gln Leu His Gln Gln Cys Cys Glu Lys Trp Thr Leu Val
    1490                1495                1500

Asn Asp Glu Thr Gln Ala Lys Met Ala Arg Met Ala Ala Ala Ala Ala
1505                1510                1515                1520

Trp Gly Leu Gly Gln Trp Asp Ser Met Glu Glu Tyr Thr Cys Met Ile
                1525                1530                1535

Pro Arg Asp Thr His Asp Gly Ala Phe Tyr Arg Ala Val Leu Ala Leu
            1540                1545                1550

His Gln Asp Leu Phe Ser Leu Ala Gln Gln Cys Ile Asp Lys Ala Arg
        1555                1560                1565
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Asp Leu Leu Asp Ala Glu Leu Thr Ala Met Ala Gly Glu Ser Tyr Ser
    1570                1575                1580

Arg Ala Tyr Gly Ala Met Val Ser Cys His Met Leu Ser Glu Leu Glu
1585                1590                1595                1600

Glu Val Ile Gln Tyr Lys Leu Val Pro Glu Arg Arg Glu Ile Ile Arg
                1605                1610                1615

Gln Ile Trp Trp Glu Arg Leu Gln Gly Cys Gln Arg Ile Val Glu Asp
            1620                1625                1630

Trp Gln Lys Ile Leu Met Val Arg Ser Leu Val Val Ser Pro His Glu
        1635                1640                1645

Asp Met Arg Thr Trp Leu Lys Tyr Ala Ser Leu Cys Gly Lys Ser Gly
    1650                1655                1660

Arg Leu Ala Leu Ala His Lys Thr Leu Val Leu Leu Leu Gly Val Asp
1665                1670                1675                1680

Pro Ser Arg Gln Leu Asp His Pro Leu Pro Thr Val His Pro Gln Val
                1685                1690                1695

Thr Tyr Ala Tyr Met Lys Asn Met Trp Lys Ser Ala Arg Lys Ile Asp
            1700                1705                1710

Ala Phe Gln His Met Gln His Phe Val Gln Thr Met Gln Gln Gln Ala
        1715                1720                1725

Gln His Ala Ile Ala Thr Glu Asp Gln Gln His Lys Gln Glu Leu His
    1730                1735                1740

Lys Leu Met Ala Arg Cys Phe Leu Lys Leu Gly Glu Trp Gln Leu Asn
1745                1750                1755                1760

Leu Gln Gly Ile Asn Glu Ser Thr Ile Pro Lys Val Leu Gln Tyr Tyr
                1765                1770                1775

Ser Ala Ala Thr Glu His Asp Arg Ser Trp Tyr Lys Ala Trp His Ala
            1780                1785                1790

Trp Ala Val Met Asn Phe Glu Ala Val Leu His Tyr Lys His Gln Asn
        1795                1800                1805

Gln Ala Arg Asp Glu Lys Lys Lys Leu Arg His Ala Ser Gly Ala Asn
    1810                1815                1820

Ile Thr Asn Ala Thr Thr Thr Ala Thr Thr Ala Ala Ser Ala Ala Ala
1825                1830                1835                1840

Ala Thr Ser Thr Glu Gly Ser Asn Ser Glu Ser Glu Ala Glu Ser Asn
                1845                1850                1855

Glu Ser Ser Pro Thr Pro Ser Pro Leu Gln Lys Lys Val Thr Glu Asp
            1860                1865                1870

Leu Ser Lys Thr Leu Leu Leu Tyr Thr Val Pro Ala Val Gln Gly Phe
        1875                1880                1885

Phe Arg Ser Ile Ser Leu Ser Arg Gly Asn Asn Leu Gln Asp Thr Leu
    1890                1895                1900

Arg Val Leu Thr Leu Trp Phe Asp Tyr Gly His Trp Pro Asp Val Asn
1905                1910                1915                1920

Glu Ala Leu Val Glu Gly Val Lys Ala Ile Gln Ile Asp Thr Trp Leu
                1925                1930                1935

Gln Val Ile Pro Gln Leu Ile Ala Arg Ile Asp Thr Pro Arg Pro Leu
            1940                1945                1950

Val Gly Arg Leu Ile His Gln Leu Leu Thr Asp Ile Gly Arg Tyr His
        1955                1960                1965

Pro Gln Ala Leu Ile Tyr Pro Leu Thr Val Ala Ser Lys Ser Thr Thr
    1970                1975                1980

Thr Ala Arg His Asn Ala Ala Asn Lys Ile Leu Lys Asn Met Cys Glu
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1985                1990                1995                2000

His Ser Asn Thr Leu Val Gln Gln Ala Met Met Val Ser Glu Glu Leu
                2005                2010                2015

Ile Arg Val Ala Ile Leu Trp His Glu Met Trp His Glu Gly Leu Glu
            2020                2025                2030

Glu Ala Ser Arg Leu Tyr Phe Gly Glu Arg Asn Val Lys Gly Met Phe
        2035                2040                2045

Glu Val Leu Glu Pro Leu His Ala Met Met Glu Arg Gly Pro Gln Thr
    2050                2055                2060

Leu Lys Glu Thr Ser Phe Asn Gln Ala Tyr Gly Arg Asp Leu Met Glu
2065                2070                2075                2080

Ala Gln Glu Trp Cys Arg Lys Tyr Met Lys Ser Gly Asn Val Lys Asp
                2085                2090                2095

Leu Thr Gln Ala Trp Asp Leu Tyr Tyr His Val Phe Arg Arg Ile Ser
            2100                2105                2110

Lys Gln Leu Pro Gln Leu Thr Ser Leu Glu Leu Gln Tyr Val Ser Pro
        2115                2120                2125

Lys Leu Leu Met Cys Arg Asp Leu Glu Leu Ala Val Pro Gly Thr Tyr
    2130                2135                2140

Asp Pro Asn Gln Thr Ile Ile Arg Ile Gln Ser Ile Ala Pro Ser Leu
2145                2150                2155                2160

Gln Val Ile Thr Ser Lys Gln Arg Pro Arg Lys Leu Thr Leu Met Gly
                2165                2170                2175

Ser Asn Gly His Glu Phe Val Phe Leu Leu Lys Gly His Glu Asp Leu
            2180                2185                2190

Arg Gln Asp Glu Arg Val Met Gln Leu Phe Gly Leu Val Asn Thr Leu
        2195                2200                2205

Leu Ala Asn Asp Pro Thr Ser Leu Arg Lys Asn Leu Ser Ile Gln Arg
    2210                2215                2220

Tyr Ala Val Ile Pro Leu Ser Thr Asn Ser Gly Leu Ile Gly Trp Val
2225                2230                2235                2240

Pro His Cys Asp Thr Leu His Ala Leu Ile Arg Asp Tyr Arg Glu Lys
                2245                2250                2255

Lys Lys Ile Leu Leu Asn Ile Glu His Arg Ile Met Leu Arg Met Ala
            2260                2265                2270

Pro Asp Tyr Asp His Leu Thr Leu Met Gln Lys Val Glu Val Phe Glu
        2275                2280                2285

His Ala Val Asn Asn Thr Ala Gly Asp Asp Leu Ala Lys Leu Leu Trp
    2290                2295                2300

Leu Lys Ser Pro Ser Ser Glu Val Trp Phe Asp Arg Arg Thr Asn Tyr
2305                2310                2315                2320

Thr Arg Ser Leu Ala Val Met Ser Met Val Gly Tyr Ile Leu Gly Leu
                2325                2330                2335

Gly Asp Arg His Pro Ser Asn Leu Met Leu Asp Arg Leu Ser Gly Lys
            2340                2345                2350

Ile Leu His Ile Asp Phe Gly Asp Cys Phe Glu Val Ala Met Thr Arg
        2355                2360                2365

Glu Lys Phe Pro Glu Lys Ile Pro Phe Arg Leu Thr Arg Met Leu Thr
    2370                2375                2380

Asn Ala Met Glu Val Thr Gly Leu Asp Arg Asn Tyr Arg Thr Thr Cys
2385                2390                2395                2400

His Thr Val Met Glu Val Leu Arg Glu His Lys Asp Ser Val Met Ala
                2405                2410                2415
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Val Leu Glu Ala Phe Val Tyr Asp Pro Leu Leu Asn Trp Arg Leu Met
            2420                2425                2430

Asp Thr Asn Ala Lys Gly Asn Lys Arg Ser Arg Thr Arg Thr Asp Ser
        2435                2440                2445

Tyr Ser Ala Gly Gln Ser Val Glu Ile Leu Asp Gly Val Glu Leu Gly
    2450                2455                2460

Glu Pro Ala His Lys Lys Thr Gly Thr Thr Val Pro Glu Ser Ile His
2465                2470                2475                2480

Ser Phe Ile Gly Asp Gly Leu Val Lys Pro Glu Ala Leu Asn Lys Lys
                2485                2490                2495

Ala Ile Gln Ile Ile Asn Arg Val Arg Asp Lys Leu Thr Gly Arg Asp
            2500                2505                2510

Phe Ser His Asp Asp Thr Leu Asp Val Pro Thr Gln Val Glu Leu Leu
        2515                2520                2525

Ile Lys Gln Ala Thr Ser His Glu Asn Leu Cys Gln Cys Tyr Ile Gly
    2530                2535                2540

Trp Cys Pro Phe Trp
2545
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2470 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
Met Glu Pro His Glu Glu Gln Ile Trp Lys Ser Lys Leu Leu Lys Ala
1               5                   10                  15

Ala Asn Asn Asp Met Asp Met Asp Arg Asn Val Pro Leu Ala Pro Asn
            20                  25                  30

Leu Asn Val Asn Met Asn Met Lys Met Asn Ala Ser Arg Asn Gly Asp
        35                  40                  45

Glu Phe Gly Leu Thr Ser Ser Arg Phe Gly Gly Val Val Ile Gly Ser
    50                  55                  60

Asn Gly Asp Val Asn Phe Lys Pro Ile Leu Glu Lys Ile Phe Arg Glu
65                  70                  75                  80

Leu Thr Ser Asp Tyr Lys Glu Glu Arg Lys Leu Ala Ser Ile Ser Leu
                85                  90                  95

Phe Asp Leu Leu Val Ser Leu Glu His Glu Leu Ser Ile Glu Glu Phe
            100                 105                 110

Gln Ala Ile Ser Asn Asp Ile Asn Asn Lys Ile Leu Glu Leu Val His
        115                 120                 125

Thr Lys Lys Thr Asn Thr Arg Val Gly Ala Val Leu Ser Ile Asp Thr
    130                 135                 140

Leu Ile Ser Phe Tyr Ala Tyr Thr Glu Arg Leu Pro Asn Glu Thr Ser
145                 150                 155                 160

Arg Leu Ala Gly Tyr Leu Arg Gly Leu Ile Pro Ser Asn Asp Val Glu
                165                 170                 175

Val Met Arg Leu Ala Ala Lys Thr Leu Gly Lys Leu Ala Val Pro Gly
            180                 185                 190
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Gly Thr Tyr Thr Ser Asp Phe Val Glu Phe Glu Ile Lys Ser Cys Leu
    195                 200                 205

Glu Trp Leu Thr Ala Ser Thr Glu Lys Asn Ser Phe Ser Ser Ser Lys
    210                 215                 220

Pro Asp His Ala Lys His Ala Ala Leu Leu Ile Ile Thr Ala Leu Ala
225                 230                 235                 240

Glu Asn Cys Pro Tyr Leu Leu Tyr Gln Tyr Leu Asn Ser Ile Leu Asp
                245                 250                 255

Asn Ile Trp Arg Ala Leu Arg Asp Pro His Leu Val Ile Arg Ile Asp
            260                 265                 270

Ala Ser Ile Thr Leu Ala Lys Cys Leu Ser Thr Leu Arg Asn Arg Asp
        275                 280                 285

Pro Gln Leu Thr Ser Gln Trp Val Gln Arg Leu Ala Thr Ser Cys Glu
    290                 295                 300

Tyr Gly Phe Gln Val Asn Thr Leu Glu Cys Ile His Ala Ser Leu Leu
305                 310                 315                 320

Val Tyr Lys Glu Ile Leu Phe Leu Lys Asp Pro Phe Leu Asn Gln Val
                325                 330                 335

Phe Asp Gln Met Cys Leu Asn Cys Ile Ala Tyr Glu Asn His Lys Ala
            340                 345                 350

Lys Met Ile Arg Glu Lys Ile Tyr Gln Ile Val Pro Leu Leu Ala Ser
        355                 360                 365

Phe Asn Pro Gln Leu Phe Ala Gly Lys Tyr Leu His Gln Ile Met Asp
    370                 375                 380

Asn Tyr Leu Glu Ile Leu Thr Asn Ala Pro Ala Lys Lys Ile Pro His
385                 390                 395                 400

Leu Lys Asp Asp Lys Pro Gln Ile Leu Ile Ser Ile Gly Asp Ile Ala
                405                 410                 415

Tyr Glu Val Gly Pro Asp Ile Ala Pro Tyr Val Lys Gln Ile Leu Asp
            420                 425                 430

Tyr Ile Glu His Asp Leu Gln Thr Lys Phe Lys Phe Arg Lys Lys Phe
        435                 440                 445

Glu Asn Glu Ile Phe Tyr Cys Ile Gly Arg Leu Ala Val Pro Leu Gly
    450                 455                 460

Pro Val Leu Gly Lys Leu Leu Asn Arg Asn Ile Leu Asp Leu Met Phe
465                 470                 475                 480

Lys Cys Pro Leu Ser Asp Tyr Met Gln Glu Thr Phe Gln Ile Leu Thr
                485                 490                 495

Glu Arg Ile Pro Ser Leu Gly Pro Lys Ile Asn Asp Glu Leu Leu Asn
            500                 505                 510

Leu Val Cys Ser Thr Leu Ser Gly Thr Pro Phe Ile Gln Pro Gly Ser
        515                 520                 525

Pro Met Glu Ile Pro Ser Phe Ser Arg Glu Arg Ala Arg Glu Trp Arg
    530                 535                 540

Asn Lys Ser Ile Leu Gln Lys Thr Gly Glu Ser Asn Asp Asp Asn Asn
545                 550                 555                 560

Asp Ile Lys Ile Ile Ile Gln Ala Phe Arg Met Leu Lys Asn Ile Lys
                565                 570                 575

Ser Arg Phe Ser Leu Val Glu Phe Val Arg Ile Val Ala Leu Ser Tyr
            580                 585                 590

Ile Glu His Thr Asp Pro Arg Val Arg Lys Leu Ala Ala Leu Thr Ser
        595                 600                 605

Cys Glu Ile Tyr Val Lys Asp Asn Ile Cys Lys Gln Thr Ser Leu His
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                610                 615                 620

Ser Leu Asn Thr Val Ser Glu Val Leu Ser Lys Leu Leu Ala Ile Thr
625                 630                 635                 640

Ile Ala Asp Pro Leu Gln Asp Ile Arg Leu Glu Val Leu Lys Asn Leu
                645                 650                 655

Asn Pro Cys Phe Asp Pro Gln Leu Ala Gln Pro Asp Asn Leu Arg Leu
            660                 665                 670

Leu Phe Thr Ala Leu His Asp Glu Ser Phe Asn Ile Gln Ser Val Ala
        675                 680                 685

Met Glu Leu Val Gly Arg Leu Ser Ser Val Asn Pro Ala Tyr Val Ile
    690                 695                 700

Pro Ser Ile Arg Lys Ile Leu Leu Glu Leu Leu Thr Lys Leu Lys Phe
705                 710                 715                 720

Ser Thr Ser Ser Arg Glu Lys Glu Glu Thr Ala Ser Leu Leu Cys Thr
                725                 730                 735

Leu Ile Arg Ser Ser Lys Asp Val Ala Lys Pro Tyr Ile Glu Pro Leu
            740                 745                 750

Leu Asn Val Leu Leu Pro Lys Phe Gln Asp Thr Ser Ser Thr Val Ala
        755                 760                 765

Ser Thr Ala Leu Arg Thr Ile Gly Glu Leu Ser Val Val Gly Gly Glu
    770                 775                 780

Asp Met Lys Ile Tyr Leu Lys Asp Leu Phe Pro Leu Ile Ile Lys Thr
785                 790                 795                 800

Phe Gln Asp Gln Ser Asn Ser Phe Lys Arg Glu Ala Ala Leu Lys Ala
                805                 810                 815

Leu Gly Gln Leu Ala Ala Ser Ser Gly Tyr Val Ile Asp Pro Leu Leu
            820                 825                 830

Asp Tyr Pro Glu Leu Leu Gly Ile Leu Val Asn Ile Leu Lys Thr Glu
        835                 840                 845

Asn Ser Gln Asn Ile Arg Arg Gln Thr Val Thr Leu Ile Gly Ile Leu
    850                 855                 860

Gly Ala Ile Asp Pro Tyr Arg Gln Lys Glu Arg Glu Val Thr Ser Thr
865                 870                 875                 880

Thr Asp Ile Ser Thr Glu Gln Asn Ala Pro Pro Ile Asp Ile Ala Leu
                885                 890                 895

Leu Met Gln Gly Met Ser Pro Ser Asn Asp Glu Tyr Tyr Thr Thr Val
            900                 905                 910

Val Ile His Cys Leu Leu Lys Ile Leu Lys Asp Pro Ser Leu Ser Ser
        915                 920                 925

Tyr His Thr Ala Val Ile Gln Ala Ile Met His Ile Phe Gln Thr Leu
    930                 935                 940

Gly Leu Lys Cys Val Ser Phe Leu Asp Gln Ile Ile Pro Thr Ile Leu
945                 950                 955                 960

Asp Val Met Arg Thr Cys Ser Gln Ser Leu Leu Glu Phe Tyr Phe Gln
                965                 970                 975

Gln Leu Cys Ser Leu Ile Ile Ile Val Arg Gln His Ile Arg Pro His
            980                 985                 990

Val Asp Ser Ile Phe Gln Ala Ile Lys Asp Phe Ser Ser Val Ala Lys
        995                 1000                1005

Leu Gln Ile Thr Leu Val Ser Val Ile Glu Ala Ile Ser Lys Ala Leu
    1010                1015                1020

Glu Gly Glu Phe Lys Arg Leu Val Pro Leu Thr Leu Thr Leu Phe Leu
1025                1030                1035                1040
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Val Ile Leu Glu Asn Asp Lys Ser Ser Asp Lys Val Leu Ser Arg Arg
            1045                1050                1055

Val Leu Arg Leu Leu Glu Ser Phe Gly Pro Asn Leu Glu Gly Tyr Ser
        1060                1065                1070

His Leu Ile Thr Pro Lys Ile Val Gln Met Ala Glu Phe Thr Ser Gly
    1075                1080                1085

Asn Leu Gln Arg Ser Ala Ile Ile Thr Ile Gly Lys Leu Ala Lys Asp
    1090                1095                1100

Val Asp Leu Phe Glu Met Ser Ser Arg Ile Val His Ser Leu Leu Arg
1105                1110                1115                1120

Val Leu Ser Ser Thr Thr Ser Asp Glu Leu Ser Lys Val Ile Met Asn
            1125                1130                1135

Thr Leu Ser Leu Leu Leu Ile Gln Met Gly Thr Ser Phe Ala Ile Phe
        1140                1145                1150

Ile Pro Val Ile Asn Glu Val Leu Met Lys Lys His Ile Gln His Thr
    1155                1160                1165

Ile Tyr Asp Asp Leu Thr Asn Arg Ile Leu Asn Asn Asp Val Leu Pro
    1170                1175                1180

Thr Lys Ile Leu Glu Ala Asn Thr Thr Asp Tyr Lys Pro Ala Glu Gln
1185                1190                1195                1200

Met Glu Ala Ala Asp Ala Gly Val Ala Lys Leu Pro Ile Asn Gln Ser
            1205                1210                1215

Val Leu Lys Ser Ala Trp Asn Ser Ser Gln Gln Arg Thr Lys Glu Asp
        1220                1225                1230

Trp Gln Glu Trp Ser Lys Arg Leu Ser Ile Gln Leu Leu Lys Glu Ser
    1235                1240                1245

Pro Ser His Ala Leu Arg Ala Cys Ser Asn Leu Ala Ser Met Tyr Tyr
    1250                1255                1260

Pro Leu Ala Lys Glu Leu Phe Asn Thr Ala Phe Ala Cys Val Trp Thr
1265                1270                1275                1280

Glu Leu Tyr Ser Gln Tyr Gln Glu Asp Leu Ile Gly Ser Leu Cys Ile
            1285                1290                1295

Ala Leu Ser Ser Pro Leu Asn Pro Pro Glu Ile His Gln Thr Leu Leu
        1300                1305                1310

Asn Leu Val Glu Phe Met Glu His Asp Asp Lys Ala Leu Pro Ile Pro
    1315                1320                1325

Thr Gln Ser Leu Gly Glu Tyr Ala Glu Arg Cys His Ala Tyr Ala Lys
    1330                1335                1340

Ala Leu His Tyr Lys Glu Ile Lys Phe Ile Lys Glu Pro Glu Asn Ser
1345                1350                1355                1360

Thr Ile Glu Ser Leu Ile Ser Ile Asn Asn Gln Leu Asn Gln Thr Asp
            1365                1370                1375

Ala Ala Ile Gly Ile Leu Lys His Ala Gln Gln His His Ser Leu Gln
        1380                1385                1390

Leu Lys Glu Thr Trp Phe Glu Lys Leu Glu Arg Trp Glu Asp Ala Leu
    1395                1400                1405

His Ala Tyr Asn Glu Arg Glu Lys Ala Gly Asp Thr Ser Val Ser Val
    1410                1415                1420

Thr Leu Gly Lys Met Arg Ser Leu His Ala Leu Gly Glu Trp Glu Gln
1425                1430                1435                1440

Leu Ser Gln Leu Ala Ala Arg Lys Trp Lys Val Ser Lys Leu Gln Thr
            1445                1450                1455
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Lys Lys Leu Ile Ala Pro Leu Ala Ala Gly Ala Arg Trp Gly Leu Gly
          1460                1465                1470

Glu Trp Asp Met Leu Glu Gln Tyr Ile Ser Val Met Lys Pro Lys Ser
      1475                1480                1485

Pro Asp Lys Glu Phe Phe Asp Ala Ile Leu Tyr Leu His Lys Asn Asp
   1490                1495                1500

Tyr Asp Asn Ala Ser Lys His Ile Leu Asn Ala Arg Asp Leu Leu Val
1505                1510                1515                1520

Thr Glu Ile Ser Ala Leu Ile Asn Glu Ser Tyr Asn Arg Ala Tyr Ser
            1525                1530                1535

Val Ile Val Arg Thr Gln Ile Ile Thr Glu Phe Glu Glu Ile Ile Lys
          1540                1545                1550

Tyr Lys Gln Leu Pro Pro Asn Ser Glu Lys Lys Leu His Tyr Gln Asn
      1555                1560                1565

Leu Trp Thr Lys Arg Leu Leu Gly Cys Gln Lys Asn Val Asp Leu Trp
   1570                1575                1580

Gln Arg Val Leu Arg Val Arg Ser Leu Val Ile Lys Pro Lys Gln Asp
1585                1590                1595                1600

Leu Gln Ile Trp Ile Lys Phe Ala Asn Leu Cys Arg Lys Ser Gly Arg
            1605                1610                1615

Met Arg Leu Ala Asn Lys Ala Leu Asn Met Leu Leu Glu Gly Gly Asn
          1620                1625                1630

Asp Pro Ser Leu Pro Asn Thr Val Lys Ala Pro Pro Pro Val Val Tyr
      1635                1640                1645

Ala Gln Leu Lys Tyr Ile Trp Ala Thr Gly Ala Tyr Lys Glu Ala Leu
   1650                1655                1660

Asn His Leu Ile Gly Phe Thr Ser Arg Leu Ala His Asp Leu Gly Leu
1665                1670                1675                1680

Asp Pro Asn Asn Met Ile Ala Gln Ser Val Lys Leu Ser Ser Ala Ser
            1685                1690                1695

Thr Ala Pro Tyr Val Glu Glu Tyr Thr Lys Leu Leu Ala Arg Cys Phe
          1700                1705                1710

Leu Lys Gln Gly Glu Trp Arg Ile Ala Thr Gln Pro Asn Trp Arg Asn
      1715                1720                1725

Thr Asn Pro Asp Ala Ile Leu Gly Ser Tyr Leu Leu Ala Thr His Phe
   1730                1735                1740

Asp Lys Asn Trp Tyr Lys Ala Trp His Asn Trp Ala Leu Ala Asn Phe
1745                1750                1755                1760

Glu Val Ile Ser Met Val Gln Glu Glu Thr Lys Leu Asn Gly Gly Lys
            1765                1770                1775

Asn Asp Asp Asp Asp Asp Thr Ala Val Asn Asn Asp Asn Val Arg Ile
          1780                1785                1790

Asp Gly Ser Ile Leu Gly Ser Gly Ser Leu Thr Ile Asn Gly Asn Arg
      1795                1800                1805

Tyr Pro Leu Glu Leu Ile Gln Arg His Val Val Pro Ala Ile Lys Gly
   1810                1815                1820

Phe Phe His Ser Ile Ser Leu Leu Glu Thr Ser Cys Leu Gln Asp Thr
1825                1830                1835                1840

Leu Arg Leu Leu Thr Leu Leu Phe Asn Phe Gly Gly Ile Lys Glu Val
            1845                1850                1855

Ser Gln Ala Met Tyr Glu Gly Phe Asn Leu Met Lys Ile Glu Asn Trp
          1860                1865                1870

Leu Glu Val Leu Pro Gln Leu Ile Ser Arg Ile His Gln Pro Asp Pro
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            1875                1880                1885

Thr Val Ser Asn Ser Leu Leu Ser Leu Leu Ser Asp Leu Gly Lys Ala
    1890                1895                1900

His Pro Gln Ala Leu Val Tyr Pro Leu Thr Val Ala Ile Lys Ser Glu
1905                1910                1915                1920

Ser Val Ser Arg Gln Lys Ala Ala Leu Ser Ile Ile Glu Lys Ile Arg
                1925                1930                1935

Ile His Ser Pro Val Leu Val Asn Gln Ala Glu Leu Val Ser His Glu
            1940                1945                1950

Leu Ile Arg Val Ala Val Leu Trp His Glu Leu Trp Tyr Glu Gly Leu
        1955                1960                1965

Glu Asp Ala Arg Arg Gln Phe Phe Val Glu His Asn Ile Glu Lys Met
    1970                1975                1980

Phe Ser Thr Leu Glu Pro Leu His Lys His Leu Gly Asn Glu Pro Gln
1985                1990                1995                2000

Thr Leu Ser Glu Val Ser Phe Gln Lys Ser Phe Gly Arg Asp Leu Asn
                2005                2010                2015

Asp Ala Tyr Glu Trp Leu Asn Asn Tyr Lys Lys Ser Lys Asp Ile Asn
            2020                2025                2030

Asn Leu Asn Gln Ala Trp Asp Ile Tyr Tyr Asn Val Phe Arg Lys Ile
        2035                2040                2045

Thr Arg Gln Ile Pro Gln Leu Gln Thr Leu Asp Leu Gln His Val Ser
    2050                2055                2060

Pro Gln Leu Leu Ala Thr His Asp Leu Glu Leu Ala Val Pro Gly Thr
2065                2070                2075                2080

Tyr Phe Pro Gly Lys Pro Thr Ile Arg Ile Ala Lys Phe Glu Pro Leu
                2085                2090                2095

Phe Ser Val Ile Ser Ser Lys Gln Arg Pro Arg Lys Phe Ser Ile Lys
            2100                2105                2110

Gly Ser Asp Gly Lys Asp Tyr Lys Tyr Val Leu Lys Gly His Glu Asp
        2115                2120                2125

Ile Arg Gln Asp Ser Leu Val Met Gln Leu Phe Gly Leu Val Asn Thr
    2130                2135                2140

Leu Leu Lys Asn Asp Ser Glu Cys Phe Lys Arg His Leu Asp Ile Gln
2145                2150                2155                2160

Gln Tyr Pro Ala Ile Pro Leu Ser Pro Lys Ser Gly Leu Leu Gly Trp
                2165                2170                2175

Val Pro Asn Ser Asp Thr Phe His Val Leu Ile Arg Glu His Arg Asp
            2180                2185                2190

Ala Lys Lys Ile Pro Leu Asn Ile Glu Gln Trp Val Met Leu Gln Met
        2195                2200                2205

Ala Pro Asp Tyr Glu Asn Leu Thr Leu Leu Gln Lys Ile Glu Val Phe
    2210                2215                2220

Thr Tyr Ala Leu Asp Asn Thr Lys Gly Gln Asp Leu Tyr Lys Ile Leu
2225                2230                2235                2240

Trp Leu Lys Ser Arg Ser Ser Glu Thr Trp Leu Glu Arg Arg Thr Thr
                2245                2250                2255

Tyr Thr Arg Ser Leu Ala Val Met Ser Met Thr Gly Tyr Ile Leu Gly
            2260                2265                2270

Leu Gly Asp Arg His Pro Ser Asn Leu Met Leu Asp Arg Ile Thr Gly
        2275                2280                2285

Lys Val Ile His Ile Asp Phe Gly Asp Cys Phe Glu Ala Ala Ile Leu
    2290                2295                2300
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Arg Glu Lys Tyr Pro Glu Lys Val Pro Phe Arg Leu Thr Arg Met Leu
2305 2310 2315 2320

Thr Tyr Ala Met Glu Val Ser Gly Ile Glu Gly Ser Phe Arg Ile Thr
2325 2330 2335

Cys Glu Asn Val Met Arg Val Leu Arg Asp Asn Lys Glu Ser Leu Met
2340 2345 2350

Ala Ile Leu Glu Ala Phe Ala Leu Asp Pro Leu Ile His Trp Gly Phe
2355 2360 2365

Asp Leu Pro Pro Gln Lys Leu Thr Glu Gln Thr Gly Ile Pro Leu Pro
2370 2375 2380

Leu Ile Asn Pro Ser Glu Leu Leu Arg Lys Gly Ala Ile Thr Val Glu
2385 2390 2395 2400

Glu Ala Ala Asn Met Glu Ala Glu Gln Gln Asn Glu Thr Arg Asn Ala
2405 2410 2415

Arg Ala Met Leu Val Leu Arg Arg Ile Thr Asp Lys Leu Thr Gly Asn
2420 2425 2430

Asp Ile Lys Arg Phe Asn Glu Leu Asp Val Pro Glu Gln Val Asp Lys
2435 2440 2445

Leu Ile Gln Gln Ala Thr Ser Ile Glu Arg Leu Cys Gln His Tyr Ile
2450 2455 2460

Gly Trp Cys Pro Phe Trp
2465 2470

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2474 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Saccharomyces cerevisiae

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Asn Lys Tyr Ile Asn Lys Tyr Thr Thr Pro Pro Asn Leu Leu Ser
1 5 10 15

Leu Arg Gln Arg Ala Glu Gly Lys His Arg Thr Arg Lys Lys Leu Thr
20 25 30

His Lys Ser His Ser His Asp Asp Glu Met Ser Thr Thr Ser Asn Thr
35 40 45

Asp Ser Asn His Asn Gly Pro Asn Asp Ser Gly Arg Val Ile Thr Gly
50 55 60

Ser Ala Gly His Ile Gly Lys Ile Ser Phe Val Asp Ser Glu Leu Asp
65 70 75 80

Thr Thr Phe Ser Thr Leu Asn Leu Ile Phe Asp Lys Leu Lys Ser Asp
85 90 95

Val Pro Gln Glu Arg Ala Ser Gly Ala Asn Glu Leu Ser Thr Thr Leu
100 105 110

Thr Ser Leu Ala Arg Glu Val Ser Ala Glu Gln Phe Gln Arg Phe Ser
115 120 125

Asn Ser Leu Asn Asn Lys Ile Phe Glu Leu Ile His Gly Phe Thr Ser
130 135 140

Ser Glu Lys Ile Gly Gly Ile Leu Ala Val Asp Thr Leu Ile Ser Phe
145 150 155 160

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Tyr Leu Ser Thr Glu Glu Leu Pro Asn Gln Thr Ser Arg Leu Ala Asn
165 170 175

Tyr Leu Arg Val Leu Ile Pro Ser Ser Asp Ile Glu Val Met Arg Leu
180 185 190

Ala Ala Asn Thr Leu Gly Arg Leu Thr Val Pro Gly Gly Thr Leu Thr
195 200 205

Ser Asp Phe Val Glu Phe Glu Val Arg Thr Cys Ile Asp Trp Leu Thr
210 215 220

Leu Thr Ala Asp Asn Asn Ser Ser Ser Ser Lys Leu Glu Tyr Arg Arg
225 230 235 240

His Ala Ala Leu Leu Ile Ile Lys Ala Leu Ala Asp Asn Ser Pro Tyr
245 250 255

Leu Leu Tyr Pro Tyr Val Asn Ser Ile Leu Asp Asn Ile Trp Val Pro
260 265 270

Leu Arg Asp Ala Lys Leu Ile Ile Arg Leu Asp Ala Ala Val Ala Leu
275 280 285

Gly Lys Cys Leu Thr Ile Ile Gln Asp Arg Asp Pro Ala Leu Gly Lys
290 295 300

Gln Trp Phe Gln Arg Leu Phe Gln Gly Cys Thr His Gly Leu Ser Leu
305 310 315 320

Asn Thr Asn Asp Ser Val His Ala Thr Leu Leu Val Phe Arg Glu Leu
325 330 335

Leu Ser Leu Lys Ala Pro Tyr Leu Arg Asp Lys Tyr Asp Asp Ile Tyr
340 345 350

Lys Ser Thr Met Lys Tyr Lys Glu Tyr Lys Phe Asp Val Ile Arg Arg
355 360 365

Glu Val Tyr Ala Ile Leu Pro Leu Leu Ala Ala Phe Asp Pro Ala Ile
370 375 380

Phe Thr Lys Lys Tyr Leu Asp Arg Ile Met Val His Tyr Leu Arg Tyr
385 390 395 400

Leu Lys Asn Ile Asp Met Asn Ala Ala Asn Asn Ser Asp Lys Pro Phe
405 410 415

Ile Leu Val Ser Ile Gly Asp Ile Ala Phe Glu Val Gly Ser Ser Ile
420 425 430

Ser Pro Tyr Met Thr Leu Ile Leu Asp Asn Ile Arg Glu Gly Leu Arg
435 440 445

Thr Lys Phe Lys Val Arg Lys Gln Phe Glu Lys Asp Leu Phe Tyr Cys
450 455 460

Ile Gly Lys Leu Ala Cys Ala Leu Gly Pro Ala Phe Ala Lys His Leu
465 470 475 480

Asn Lys Asp Leu Leu Asn Leu Met Leu Asn Cys Pro Met Ser Asp His
485 490 495

Met Gln Glu Thr Leu Met Ile Leu Asn Glu Lys Ile Pro Ser Leu Glu
500 505 510

Ser Thr Val Asn Ser Arg Ile Leu Asn Leu Leu Ser Ile Ser Leu Ser
515 520 525

Gly Glu Lys Phe Ile Gln Ser Asn Gln Tyr Asp Phe Asn Asn Gln Phe
530 535 540

Ser Ile Glu Lys Ala Arg Lys Ser Arg Asn Gln Ser Phe Met Lys Lys
545 550 555 560

Thr Gly Glu Ser Asn Asp Asp Ile Thr Asp Ala Gln Ile Leu Ile Gln
565 570 575

Cys Phe Lys Met Leu Gln Leu Ile His His Gln Tyr Ser Leu Thr Glu

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            580                   585                   590

Phe Val Arg Leu Ile Thr Ile Ser Tyr Ile Glu His Glu Asp Ser Ser
        595                 600                 605

Val Arg Lys Leu Ala Ala Leu Thr Ser Cys Asp Leu Phe Ile Lys Asp
    610                 615                 620

Asp Ile Cys Lys Gln Thr Ser Val His Ala Leu His Ser Val Ser Glu
625                 630                 635                 640

Val Leu Ser Lys Leu Leu Met Ile Ala Ile Thr Asp Pro Val Ala Glu
                645                 650                 655

Ile Arg Leu Glu Ile Leu Gln His Leu Gly Ser Asn Phe Asp Pro Gln
            660                 665                 670

Leu Ala Gln Pro Asp Asn Leu Arg Leu Leu Phe Met Ala Leu Asn Asp
        675                 680                 685

Glu Ile Phe Gly Ile Gln Leu Glu Ala Ile Lys Ile Ile Gly Arg Leu
    690                 695                 700

Ser Ser Val Asn Pro Ala Tyr Val Val Pro Ser Leu Arg Lys Thr Leu
705                 710                 715                 720

Leu Glu Leu Leu Thr Gln Leu Lys Phe Ser Asn Met Pro Lys Lys Lys
                725                 730                 735

Glu Glu Ser Ala Thr Leu Leu Cys Thr Leu Ile Asn Ser Ser Asp Glu
            740                 745                 750

Val Ala Lys Pro Tyr Ile Asp Pro Ile Leu Asp Val Ile Leu Pro Lys
        755                 760                 765

Cys Gln Asp Ala Ser Ser Ala Val Ala Ser Thr Ala Leu Lys Val Leu
    770                 775                 780

Gly Glu Leu Ser Val Val Gly Gly Lys Glu Met Thr Arg Tyr Leu Lys
785                 790                 795                 800

Glu Leu Met Pro Leu Ile Ile Asn Thr Phe Gln Asp Gln Ser Asn Ser
                805                 810                 815

Phe Lys Arg Asp Ala Ala Leu Thr Thr Leu Gly Gln Leu Ala Ala Ser
            820                 825                 830

Ser Gly Tyr Val Val Gly Pro Leu Leu Asp Tyr Pro Glu Leu Leu Gly
        835                 840                 845

Ile Leu Ile Asn Ile Leu Lys Thr Glu Asn Asn Pro His Ile Arg Arg
    850                 855                 860

Gly Thr Val Arg Leu Ile Gly Ile Leu Gly Ala Leu Asp Pro Tyr Lys
865                 870                 875                 880

His Arg Glu Ile Glu Val Thr Ser Asn Ser Lys Ser Ser Val Glu Gln
                885                 890                 895

Asn Ala Pro Ser Ile Asp Ile Ala Leu Leu Met Gln Gly Val Ser Pro
            900                 905                 910

Ser Asn Asp Glu Tyr Tyr Pro Thr Val Val Ile His Asn Leu Met Lys
        915                 920                 925

Ile Leu Asn Asp Pro Ser Leu Ser Ile His His Thr Ala Ala Ile Gln
    930                 935                 940

Ala Ile Met His Ile Phe Gln Asn Leu Gly Leu Arg Cys Val Ser Phe
945                 950                 955                 960

Leu Asp Gln Ile Ile Pro Gly Ile Ile Leu Val Met Arg Ser Cys Pro
                965                 970                 975

Pro Ser Gln Leu Asp Phe Tyr Phe Gln Gln Leu Gly Ser Leu Ile Ser
            980                 985                 990

Ile Val Lys Gln His Ile Arg Pro His Val Glu Lys Ile Tyr Gly Val
        995                 1000                1005
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Ile Arg Glu Phe Phe Pro Ile Ile Lys Leu Gln Ile Thr Ile Ile Ser
    1010                1015                1020

Val Ile Glu Ser Ile Ser Lys Ala Leu Glu Gly Glu Phe Lys Arg Phe
1025                1030                1035                1040

Val Pro Glu Thr Leu Thr Phe Phe Leu Asp Ile Leu Glu Asn Asp Gln
                1045                1050                1055

Ser Asn Lys Arg Ile Val Pro Ile Arg Ile Leu Lys Ser Leu Val Thr
            1060                1065                1070

Phe Gly Pro Asn Leu Glu Asp Tyr Ser His Leu Ile Met Pro Ile Val
        1075                1080                1085

Val Arg Met Thr Glu Tyr Ser Ala Gly Ser Leu Lys Lys Ile Ser Ile
    1090                1095                1100

Ile Thr Leu Gly Arg Leu Ala Lys Asn Ile Asn Leu Ser Glu Met Ser
1105                1110                1115                1120

Ser Arg Ile Val Gln Ala Leu Val Arg Ile Leu Asn Asn Gly Asp Arg
                1125                1130                1135

Glu Leu Thr Lys Ala Thr Met Asn Thr Leu Ser Leu Leu Leu Leu Gln
            1140                1145                1150

Leu Gly Thr Asp Phe Val Val Phe Val Pro Val Ile Asn Lys Ala Leu
        1155                1160                1165

Leu Arg Asn Arg Ile Gln His Ser Val Tyr Asp Gln Leu Val Asn Lys
    1170                1175                1180

Leu Leu Asn Asn Glu Cys Leu Pro Thr Asn Ile Ile Phe Asp Lys Glu
1185                1190                1195                1200

Asn Glu Val Pro Glu Arg Lys Asn Tyr Glu Asp Glu Met Gln Val Thr
                1205                1210                1215

Lys Leu Pro Val Asn Gln Asn Ile Leu Lys Asn Ala Trp Tyr Cys Ser
            1220                1225                1230

Gln Gln Lys Thr Lys Glu Asp Trp Gln Glu Trp Ile Arg Arg Leu Ser
        1235                1240                1245

Ile Gln Leu Leu Lys Glu Ser Pro Ser Ala Cys Leu Arg Ser Cys Ser
    1250                1255                1260

Ser Leu Val Ser Val Tyr Tyr Pro Leu Ala Arg Glu Leu Phe Asn Ala
1265                1270                1275                1280

Ser Phe Ser Ser Cys Trp Val Glu Leu Gln Thr Ser Tyr Gln Glu Asp
                1285                1290                1295

Leu Ile Gln Ala Leu Cys Lys Ala Leu Ser Ser Ser Glu Asn Pro Pro
            1300                1305                1310

Glu Ile Tyr Gln Met Leu Leu Asn Leu Val Glu Phe Met Glu His Asp
        1315                1320                1325

Asp Lys Pro Leu Pro Ile Pro Ile His Thr Leu Gly Lys Tyr Ala Gln
    1330                1335                1340

Lys Cys His Ala Phe Ala Lys Ala Leu His Tyr Lys Glu Val Glu Phe
1345                1350                1355                1360

Leu Glu Glu Pro Lys Asn Ser Thr Ile Glu Ala Leu Ile Ser Ile Asn
                1365                1370                1375

Asn Gln Leu His Gln Thr Asp Ser Ala Ile Gly Ile Leu Lys His Ala
            1380                1385                1390

Gln Gln His Asn Glu Leu Gln Leu Lys Glu Thr Trp Tyr Glu Lys Leu
        1395                1400                1405

Gln Arg Trp Glu Asp Ala Leu Ala Ala Tyr Asn Glu Lys Glu Ala Ala
    1410                1415                1420
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Gly Glu Asp Ser Val Glu Val Met Met Gly Lys Leu Arg Ser Leu Tyr
1425 1430 1435 1440

Ala Leu Gly Glu Trp Glu Glu Leu Ser Lys Leu Ala Ser Glu Lys Trp
1445 1450 1455

Gly Thr Ala Lys Pro Glu Val Lys Lys Ala Met Ala Pro Leu Ala Ala
1460 1465 1470

Gly Ala Ala Trp Gly Leu Glu Gln Trp Asp Glu Ile Ala Gln Tyr Thr
1475 1480 1485

Ser Val Met Lys Ser Gln Ser Pro Asp Lys Glu Phe Tyr Asp Ala Ile
1490 1495 1500

Leu Cys Leu His Arg Asn Asn Phe Lys Lys Ala Glu Val His Ile Phe
1505 1510 1515 1520

Asn Ala Arg Asp Leu Leu Val Thr Glu Leu Ser Ala Leu Val Asn Glu
1525 1530 1535

Ser Tyr Asn Arg Ala Tyr Asn Val Val Val Arg Ala Gln Ile Ile Ala
1540 1545 1550

Glu Leu Glu Glu Ile Ile Lys Tyr Lys Lys Leu Pro Gln Asn Ser Asp
1555 1560 1565

Lys Arg Leu Thr Met Arg Glu Thr Trp Asn Thr Arg Leu Leu Gly Cys
1570 1575 1580

Gln Lys Asn Ile Asp Val Trp Gln Arg Ile Leu Arg Val Arg Ser Leu
1585 1590 1595 1600

Val Ile Lys Pro Lys Glu Asp Ala Gln Val Arg Ile Lys Phe Ala Asn
1605 1610 1615

Leu Cys Arg Lys Ser Gly Arg Met Ala Leu Ala Lys Lys Val Leu Asn
1620 1625 1630

Thr Leu Leu Glu Glu Thr Asp Asp Pro Asp His Pro Asn Thr Ala Lys
1635 1640 1645

Ala Ser Pro Pro Val Val Tyr Ala Gln Leu Lys Tyr Leu Trp Ala Thr
1650 1655 1660

Gly Leu Gln Asp Glu Ala Leu Lys Gln Leu Ile Asn Phe Thr Ser Arg
1665 1670 1675 1680

Met Ala His Asp Leu Gly Leu Asp Pro Asn Asn Met Ile Ala Gln Ser
1685 1690 1695

Val Pro Gln Gln Ser Lys Arg Val Pro Arg His Val Glu Asp Tyr Thr
1700 1705 1710

Lys Leu Leu Ala Arg Cys Phe Leu Lys Gln Gly Glu Trp Arg Val Cys
1715 1720 1725

Leu Gln Pro Lys Trp Arg Leu Ser Asn Pro Asp Ser Ile Leu Gly Ser
1730 1735 1740

Tyr Leu Leu Ala Thr His Phe Asp Asn Thr Trp Tyr Lys Ala Trp His
1745 1750 1755 1760

Asn Trp Ala Leu Ala Asn Phe Glu Val Ile Ser Met Leu Thr Ser Val
1765 1770 1775

Ser Lys Lys Lys Gln Glu Gly Ser Asp Ala Ser Ser Val Thr Asp Ile
1780 1785 1790

Asn Glu Phe Asp Asn Gly Met Ile Gly Val Asn Thr Phe Asp Ala Lys
1795 1800 1805

Glu Val His Tyr Ser Ser Asn Leu Ile His Arg His Val Ile Pro Ala
1810 1815 1820

Ile Lys Gly Phe Phe His Ser Ile Ser Leu Ser Glu Ser Ser Ser Leu
1825 1830 1835 1840

Gln Asp Ala Leu Arg Leu Leu Thr Leu Trp Phe Thr Phe Gly Gly Ile

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            1845                1850                1855

Pro Glu Ala Thr Gln Ala Met His Glu Gly Phe Asn Leu Ile Gln Ile
        1860                1865                1870

Gly Thr Trp Leu Glu Val Leu Pro Gln Leu Ile Ser Arg Ile His Gln
    1875                1880                1885

Pro Asn Gln Ile Val Ser Arg Ser Leu Leu Ser Leu Leu Ser Asp Leu
    1890                1895                1900

Gly Lys Ala His Pro Gln Ala Leu Val Tyr Pro Leu Met Val Ala Ile
1905                1910                1915                1920

Lys Ser Glu Ser Leu Ser Arg Gln Lys Ala Ala Leu Ser Ile Ile Glu
            1925                1930                1935

Lys Met Arg Ile His Ser Pro Val Leu Val Asp Gln Ala Glu Leu Val
        1940                1945                1950

Ser His Glu Leu Ile Arg Met Ala Val Leu Trp His Glu Gln Trp Tyr
    1955                1960                1965

Glu Gly Leu Asp Asp Ala Ser Arg Gln Phe Phe Gly Glu His Asn Thr
    1970                1975                1980

Glu Lys Met Phe Ala Ala Leu Glu Pro Leu Tyr Glu Met Leu Lys Arg
1985                1990                1995                2000

Gly Pro Glu Thr Leu Arg Glu Ile Ser Phe Gln Asn Ser Phe Gly Arg
            2005                2010                2015

Asp Leu Asn Asp Ala Tyr Glu Trp Leu Met Asn Tyr Lys Lys Ser Lys
        2020                2025                2030

Asp Val Ser Asn Leu Asn Gln Ala Trp Asp Ile Tyr Tyr Asn Val Phe
    2035                2040                2045

Arg Lys Ile Gly Lys Gln Leu Pro Gln Leu Gln Thr Leu Glu Leu Gln
    2050                2055                2060

His Val Ser Pro Lys Leu Leu Ser Ala His Asp Leu Glu Leu Ala Val
2065                2070                2075                2080

Pro Gly Thr Arg Ala Ser Gly Gly Lys Pro Ile Val Lys Ile Ser Lys
            2085                2090                2095

Phe Glu Pro Val Phe Ser Val Ile Ser Ser Lys Gln Arg Pro Arg Lys
        2100                2105                2110

Phe Cys Ile Lys Gly Ser Asp Gly Lys Asp Tyr Lys Tyr Val Leu Lys
    2115                2120                2125

Gly His Glu Asp Ile Arg Gln Asp Ser Leu Val Met Gln Leu Phe Gly
    2130                2135                2140

Leu Val Asn Thr Leu Leu Gln Asn Asp Ala Glu Cys Phe Arg Arg His
2145                2150                2155                2160

Leu Asp Ile Gln Gln Tyr Pro Ala Ile Pro Leu Ser Pro Lys Ser Gly
            2165                2170                2175

Leu Leu Gly Trp Val Pro Asn Ser Asp Thr Phe His Val Leu Ile Arg
        2180                2185                2190

Glu His Arg Glu Ala Lys Lys Ile Pro Leu Asn Ile Glu His Trp Val
    2195                2200                2205

Met Leu Gln Met Ala Pro Asp Tyr Asp Asn Leu Thr Leu Leu Gln Lys
    2210                2215                2220

Val Glu Val Phe Thr Tyr Ala Leu Asn Asn Thr Glu Gly Gln Asp Leu
2225                2230                2235                2240

Tyr Lys Val Leu Trp Leu Lys Ser Arg Ser Ser Glu Thr Trp Leu Glu
            2245                2250                2255

Arg Arg Thr Thr Tyr Thr Arg Ser Leu Ala Val Met Ser Met Thr Gly
        2260                2265                2270
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Tyr Ile Leu Gly Leu Gly Asp Arg His Pro Ser Asn Leu Met Leu Asp
        2275                2280                2285

Arg Ile Thr Gly Lys Val Ile His Ile Asp Phe Gly Asp Cys Phe Glu
    2290                2295                2300

Ala Ala Ile Leu Arg Glu Lys Phe Pro Glu Lys Val Pro Phe Arg Leu
2305                2310                2315                2320

Thr Arg Met Leu Thr Tyr Ala Met Glu Val Ser Gly Ile Glu Gly Ser
                2325                2330                2335

Phe Arg Ile Thr Cys Glu Asn Val Met Lys Val Leu Arg Asp Asn Lys
            2340                2345                2350

Gly Ser Leu Met Ala Ile Leu Glu Ala Phe Ala Phe Asp Pro Leu Ile
        2355                2360                2365

Asn Trp Gly Phe Asp Leu Pro Thr Lys Lys Ile Glu Glu Glu Thr Gly
    2370                2375                2380

Ile Gln Leu Pro Val Met Asn Ala Asn Glu Leu Leu Ser Asn Gly Ala
2385                2390                2395                2400

Ile Thr Glu Glu Glu Val Gln Arg Val Glu Asn Glu His Lys Asn Ala
                2405                2410                2415

Ile Arg Asn Ala Arg Ala Met Leu Val Leu Lys Arg Ile Thr Asp Lys
            2420                2425                2430

Leu Thr Gly Asn Asp Ile Arg Arg Phe Asn Asp Leu Asp Val Pro Glu
        2435                2440                2445

Gln Val Asp Lys Leu Ile Gln Gln Ala Thr Ser Val Glu Asn Leu Cys
    2450                2455                2460

Gln His Tyr Ile Gly Trp Cys Pro Phe Trp
2465                2470
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CCGGATCCCG TCGAGCTTCA GTTGAACTAC GGCGTGCTTC TGTAGCCATG GGAGTGCAGG     60

TGGA                                                                  64
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
GGCCGGAATT CTCATTCCAG TTTTAGAA                                        28
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr Tyr Asp Pro Asn Gln Pro
1 5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

His Ile Asp Phe Gly Asp
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asn Asp Gln Val Phe Glu
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GAGCCACCAC GATTTGCT 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 64 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCGGATCCCG TCGAGCTTCA GTTGAACTAC GGCGTGCTTC TGTAGCCATG GCGGCGGCCG 60

TTCC 64

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

-continued

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCCGGAATT CTCAATCAAT ATCCACTA 28

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGGGGATCCA CNTAYGAYCC NAAYCARC 28

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCGGAATTCR TCNCCRAART CDATRTG 27

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGGGATCCA AYGAYCARGT NTTYGA 26

What is claimed is:

1. An isolated, purified cDNA molecule which encodes RAFT1, a protein having the amino acid sequence as shown in SEQ ID NO:2 wherein the acronym RAFT connotes a rapamycin and FKBP12 target.

2. The isolated, purified cDNA molecule of claim 1 which comprises the nucleotide sequence as shown in SEQ ID NO:1, nucleotides 64–7710.

3. An isolated, purified intron-free DNA molecule consisting of at least 20 contiguous nucleotides encoding all or a portion of the amino acid sequence as shown in SEQ ID NO: 2.

4. An isolated, purified intron-free DNA molecule consisting of at least 20 contiguous nucleotides of the sequence as shown in SEQ ID NO: 1.

5. An isolated DNA molecule encoding a rat RAFT protein obtained by a method comprising the steps of:

(a) probing a library of rat cDNA sequences with a probe which comprises at least 15 contiguous nucleotides selected from the sequence shown in SEQ ID NO: 1; and

(b) isolating a rat cDNA molecule which (i) hybridizes to the probe, (ii) contains a complete open reading frame encoding a polypeptide of about 2550 amino acids, and (iii) encodes a rat RAFT protein,

wherein said rat RAFT protein binds to FKBP12 in the presence of 1 to 10 nM rapamycin but not in the absence of 1 to 10 nM rapamycin.

6. An isolated DNA molecule encoding a rat RAFT protein obtained by a method comprising the steps of:

(a) amplifying a DNA sequence using (i) at least one primer which comprises at least 10 contiguous nucleotides selected from the sequence shown in SEQ ID NO: 1 and (ii) a template which comprises rat cDNA or mRNA; and

(b) isolating an amplified DNA sequence which contains a complete open reading frame encoding a polypeptide of about 2550 amino acids encoding a rat RAFT protein,

wherein said rat RAFT protein binds to FKBP12 in the presence of 1 to 10 nM rapamycin but not in the absence of 1 to 10 nM rapamycin.

7. An isolated DNA molecule encoding a rat RAFT protein identified by a process comprising the steps of:

(a) annealing a set of mixed oligonucleotides to a rat cDNA library, each member of said set of mixed oligonucleotides encoding a sequence of at least six contiguous amino acids of the amino acid sequence shown in SEQ ID NO:2; and

(b) isolating a rat cDNA molecule which (i) anneals to at least one member of the set of mixed oligonucleotides, (ii) contains a complete open reading frame encoding a polypeptide of about 2550 amino acids, and (iii) encodes a rat RAFT protein,

wherein said RAFT protein binds to FKBP12 in the presence of 1 to 10 nM rapamycin but not in the absence of 1 to 10 nM rapamycin.

8. An isolated DNA molecule encoding a rat RAFT protein according to claim 7, wherein two sets of mixed oligonucleotides are annealed.

9. An isolated DNA molecule having a nucleotide sequence, or a degenerate sequence thereof, obtained by a method comprising the steps of:

(a) probing a library of rat cDNA molecules with a probe which comprises at least 15 contiguous nucleotides selected from the sequence shown in SEQ ID NO: 1; and

(b) isolating a rat cDNA molecule which (i) hybridizes to the probe, (ii) contains a complete open reading frame

encoding a polypeptide of about 2550 amino acids, and (iii) encodes a rat RAFT protein,

wherein said RAFT protein binds to FKBP12 in the presence of 1 to 10 nM rapamycin but not in the absence of 1 to 10 nM rapamycin.

10. A method of isolating a DNA molecule encoding a mammalian RAFT protein comprising the steps of:

(a) probing a library of rat cDNA sequences with a probe which comprises at least 15 contiguous nucleotides selected from the sequence shown in SEQ ID NO: 1; and

(b) isolating a rat cDNA molecule which (i) hybridizes to the probe, (ii) contains a complete open reading frame encoding a polypeptide of about 2550 amino acids, and (iii) encodes a rat RAFT protein,

wherein said rat RAFT protein binds to FKBP12 in the presence of 1 to 10 nM rapamycin but not in the absence of 1 to 10 nM rapamycin.

11. The method of claim 10 wherein the probe comprises at least 20 contiguous nucleotides encoding all or a portion of the amino acid sequence as shown in SEQ ID NO:2.

12. The method of claim 10 wherein the probe comprises at least 20 contiguous nucleotides as shown in SEQ ID NO:1.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,492,106 B1
APPLICATION NO. : 08/305790
DATED : December 10, 2002
INVENTOR(S) : David M. Sabatini et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On Title Page, should read, under (73) Assignee:

--Sloan-Kettering Institute for Cancer Research, New York, NY (US)-- has been inserted.

Signed and Sealed this

Seventeenth Day of October, 2006

Jon W. Dudas

JON W. DUDAS
Director of the United States Patent and Trademark Office